For Reference

NOT TO BE TAKEN FROM THIS ROOM

For Reference

NOT TO BE TAKEN FROM THIS ROOM

Ex libris
universitatis
albertaensis

UNIVERSITY OF ALBERTA
LIBRARY

Regulations Regarding Theses and Dissertations

Typescript copies of theses and dissertations for Master's and Doctor's degrees deposited in the University of Alberta Library, as the official Copy of the Faculty of Graduate Studies, may be consulted in the Reference Reading Room only.

A second copy is on deposit in the Department under whose supervision the work was done. Some Departments are willing to loan their copy to libraries, through the inter-library loan service of the University of Alberta Library.

These theses and dissertations are to be used only with due regard to the rights of the author. Written permission of the author and of the Department must be obtained through the University of Alberta Library when extended passages are copied. When permission has been granted, acknowledgement must appear in the published work.

This thesis or dissertation has been used in accordance with the above regulations by the persons listed below. The borrowing library is obligated to secure the signature of each user.

Please sign below:

Date	Signature	Institution

THE UNIVERSITY OF ALBERTA

SOME NEW LYCOPODIUM ALKALOIDS

by

© BRIAN ALTENKIRK

B.Sc. (Hons.), M.Sc. (Rand)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA

SPRING, 1969

Thesis
1969
1D

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled SOME NEW LYCOPODIUM ALKALOIDS submitted by BRIAN ALTENKIRK B.Sc. (Hons.), M.Sc. (Rand) in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[redacted]

Date

ACKNOWLEDGEMENTS

The author wishes to thank:

The technical staff members of the Department of Chemistry and especially Messrs. R. N. Swindlehurst and J. Hoyle for the optical spectra measurements, Messrs. G. Bigam and D. G. Gifford for the nuclear magnetic resonance spectra, Dr. A. M. Hogg and Messrs. A. I. Budd and J. Olekszyk for the determination of the mass spectra.

The National Research Council of Canada and the University of Alberta for financial assistance.

The staff members of the Department of Chemistry, University of Alberta, for their valuable assistance and encouragement.

Dr. L. Marion of the University of Ottawa and Dr. R. H. Burnell of Laval University for samples of several alkaloids.

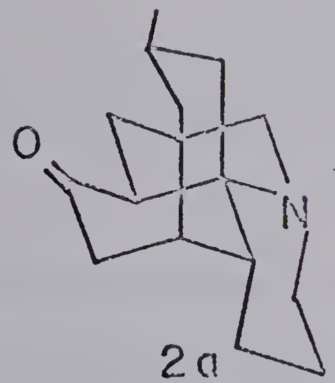
My colleagues and especially Mr. L. F. Ball and Dr. P. Smith for their help in proofreading this manuscript.

Mr. J. F. McCutcheon for his technical assistance and for his help in preparing this manuscript.

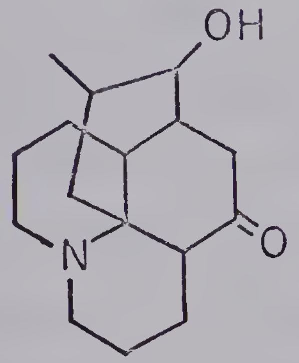
Especially, Prof. W. A. Ayer for the patient and excellent supervision which he always provided and for the wisdom and knowledge which he imparted to me.

ABSTRACT

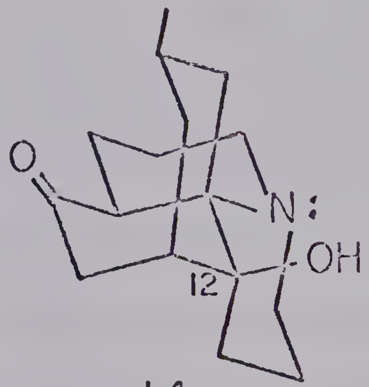
In the first part of this thesis the investigation of the alkaloids of *L. alopecuroides* L. is described. A total of ten alkaloids (2a, 3, 14a, 25, 39, 71, 78a, 80, 87a, and 89a) were isolated and characterized. These included lycopodine (2a), clavolonine (3), and lycodoline (14a). The structures of these three alkaloids have been previously determined. Anhydrolycodoline (25) was also isolated. The natural occurence of anhydrolycodoline had not been noted previously.



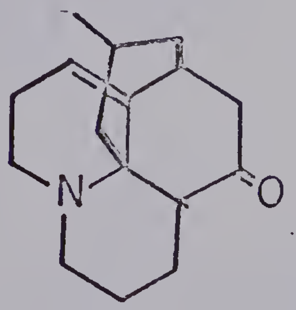
2a



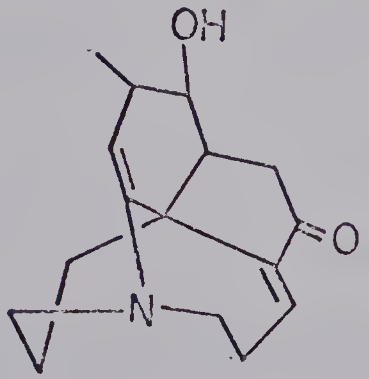
3



14a



25

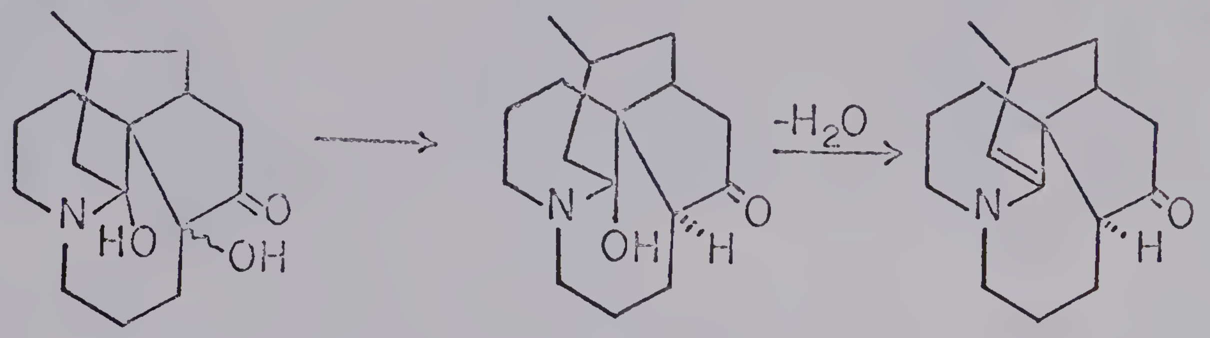


78a

The structure of alolycopine (78a) was established by physical and chemical methods.

Structures are proposed for fawcettidine (39), alopecuridine (71) and fawcettimine (38). The latter alkaloid is found in

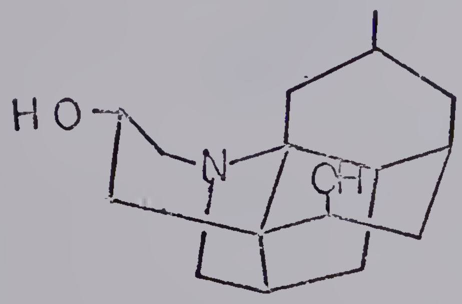
L. fawcettii. These structural assignments are based on chemical correlations and other data. The complete structures of fawcettidine



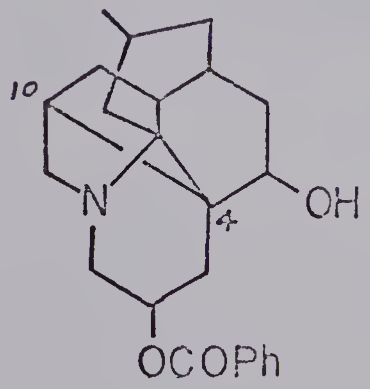
71 38 39

(39) and fawcettimine (38) are now known. The position of the hydroxyl group in alopecuridine (71) is tentatively assigned to C-4.

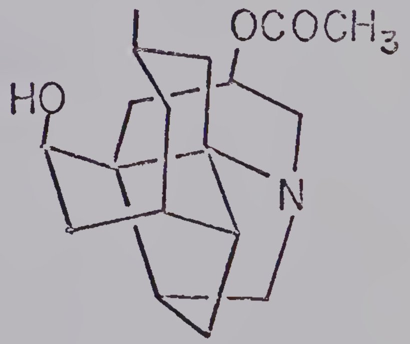
The structure of debenzoyllalopecurine (87a) was established by X-ray analysis. This enabled the structures of alopecurine (80) and acetyldebenzoylalopecurine (89) to be elucidated by chemical and physical methods.



87a

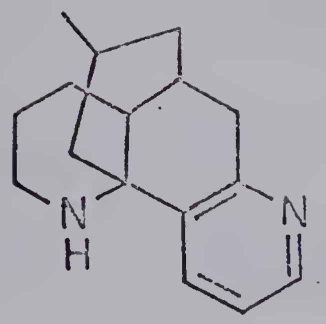


80

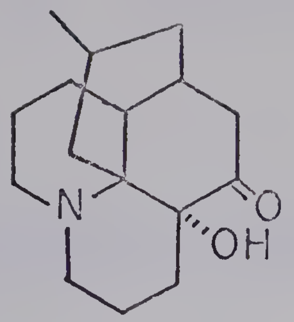


89a

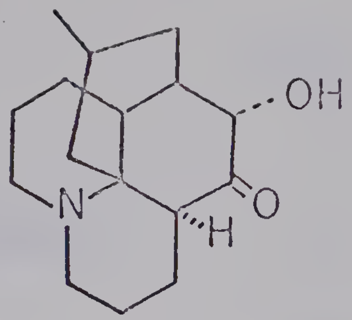
In the second part of this thesis the investigation of the strong bases of L. Lucidulum Michx. is described. A total of seven alkaloids (2a, 14a, 5, 91, 18, 98a and 119a) were isolated and characterized. These included lycopodine (2a), lycodoline (14a), lycodine (5), flabelliformine (91) and alkaloid L.20 (18) of known structure.



5

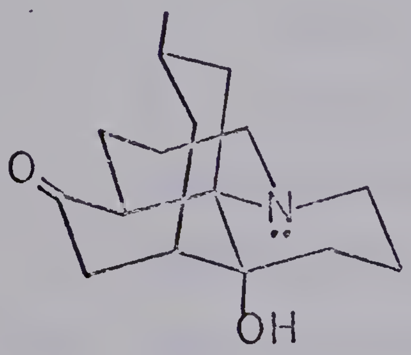


91

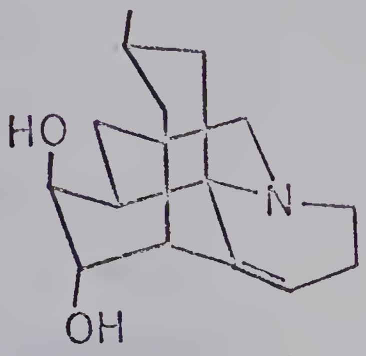


18

The structures of alkaloid L.23 (98a) and lucidioline (119a) were elucidated using chemical and physical methods.



98a



119a

TABLE OF CONTENTS

Page

INTRODUCTION 1

PART I: THE ALKALOIDS OF L. ALOPECUROIDES
Discussion and Results 16

1. Isolation 16

2(a). Structure of Fawcettidine 21

2(b). Structure of Fawcettimine 30

2(c). Structure of Alopecuridine 35

3. Structure of Alolycopine 49

4. Structure of Alopecurine 53

EXPERIMENTAL, GENERAL 63

EXPERIMENTAL, PART I 65

PART II: THE ALKALOIDS OF L. LUCIDULUM
Discussion and Results 91

1. Isolation 91

2(a). Structure of Alkaloid L.23 95

2(b). Absolute Configuration of Alkaloid L.23 104

3. Structure of Lucidioline 113

EXPERIMENTAL, PART II 125

BIBLIOGRAPHY 138

APPENDIX 1: Correspondence with Prof. Y. Inubushi 145

APPENDIX 2: Alkaloids from L. Phyllanthum 146

APPENDIX 3: Alkaloids from Unnamed Lycopodium species (Peru). 147

LIST OF FIGURES

		Page
1.	Infrared spectrum of Fawcettimine	31
2.	Infrared spectrum of N-Acetylfawcettimine	31
3.	Infrared spectrum of Alopecuridine	36
4.	Infrared spectrum of Alopecuridine	36
5.	Infrared spectrum of Alopecuridine	36
6.	Infrared spectrum of N-Acetylalopecuridine	37
7(a).	Mass spectrum of Fawcettidine	40
7(b).	Mass spectrum of Fawcettimine	40
7(c).	Mass spectrum of Alopecuridine	40
8(a).	Mass spectrum of N-Acetylfawcettimine	47
8(b).	Mass spectrum of N-Acetylalopecuridine	47
9.	Infrared spectrum of O-Acetylalolycopine	50
10.	Nuclear magnetic resonance spectrum of O-Acetylalolycopine	50
11.	Infrared spectrum of Alolycopine	50
12.	Nuclear magnetic resonance spectrum of Alopecurine	57
13.	Nuclear magnetic resonance spectrum of Acetyldebenzoylalopecurine	62
14(a).	Mass spectrum of Alkaloid L.23	104
14(b).	Mass spectrum of Lycodoline	104
15(a).	ORD spectrum of Lycopodine	108
15(b).	ORD spectrum of Lycopodine·HCl	108

Page

15(c). ORD spectrum of Lycodoline 108

15(d). ORD spectrum of Lycodoline·HCl 108

15(e). ORD spectrum of Alkaloid L.23 108

15(f). ORD spectrum of Alkaloid L.23·HCl 108

16(a). CD spectrum of Lycopodine 110

16(b). CD spectrum of Lycopodine·HCl 110

16(c). CD spectrum of Alkaloid L.23 110

16(d). CD spectrum of Alkaloid L.23·HCl 110

17(a). CD spectrum of Flabelliformine 114

17(b). CD spectrum of Flabelliformine·HCl 114

18(a). ORD spectrum of Flabelliformine 114

18(b). ORD spectrum of Flabelliformine·HCl 114

19. Mass spectrum of Lucidioline 123

LIST OF TABLES

Page

Table I 2

Table II 66

Table III 100

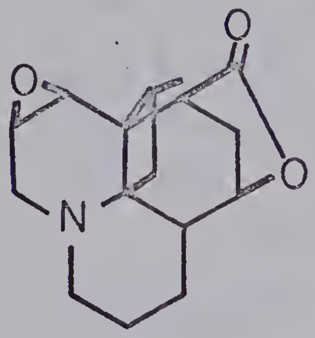
Table IV 105

LIST OF SCHEMES

	Page
Scheme I	4
Scheme II	7
Scheme III	9
Scheme IV	10
Scheme V	12
Scheme VI	48
Scheme VII	103

INTRODUCTION

The history of the *Lycopodium* alkaloids dates back to 1881 when Bödeker [1] reported the isolation of a base from *L. complanatum L.* Manske and Marion, in a series of publications between 1942 and 1953, reported the isolation of 35 bases from a variety of *Lycopodium* species and so demonstrated that this family is a rich source of alkaloids [2]. At the present time in excess of 90 alkaloids have been characterized from this family [3]. Annotinine (1), the major alkaloid of *L. annotinum L.*, was the first of these alkaloids to yield to structural studies [4]. The structure and stereochemistry of this compound were confirmed by X-ray analysis [5]. In 1960, lycopodine, the most widespread alkaloid found in the *Lycopodiaceae*, was assigned structure 2 [6]. Following this publication a whole series of structures were elucidated. Many of these structures have a similar carbon skeleton to lycopodine (2) while others differ substantially from that structure. Some representatives of the various groups are shown in table I.


Annotinine
1

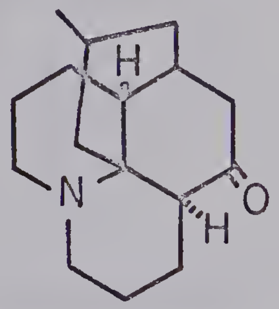
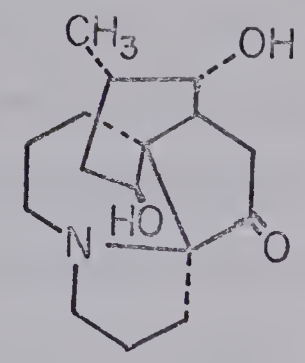
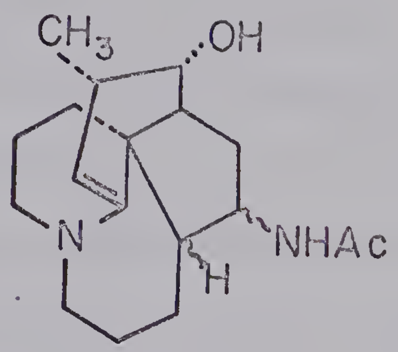

Lycopodine
2

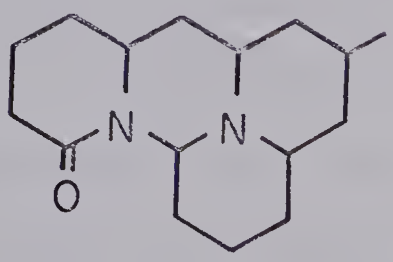
Table I



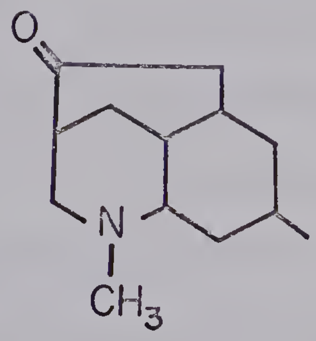
Serratinine[7]


CH3
OH
N
NHAc
H

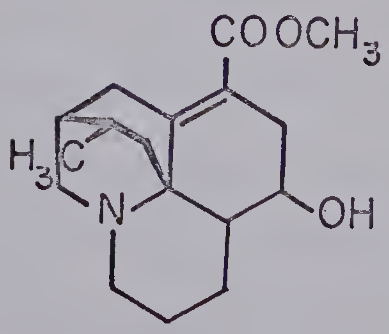
Serratinidine[8]



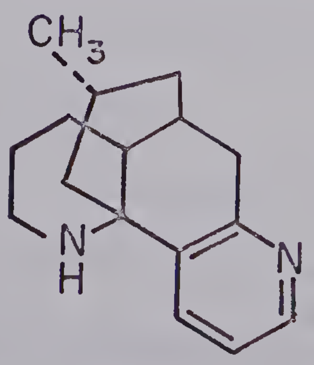
Cernuine[9]



Luciduline[10]


COOCH3
H3C
N
OH

Annopodine[11]

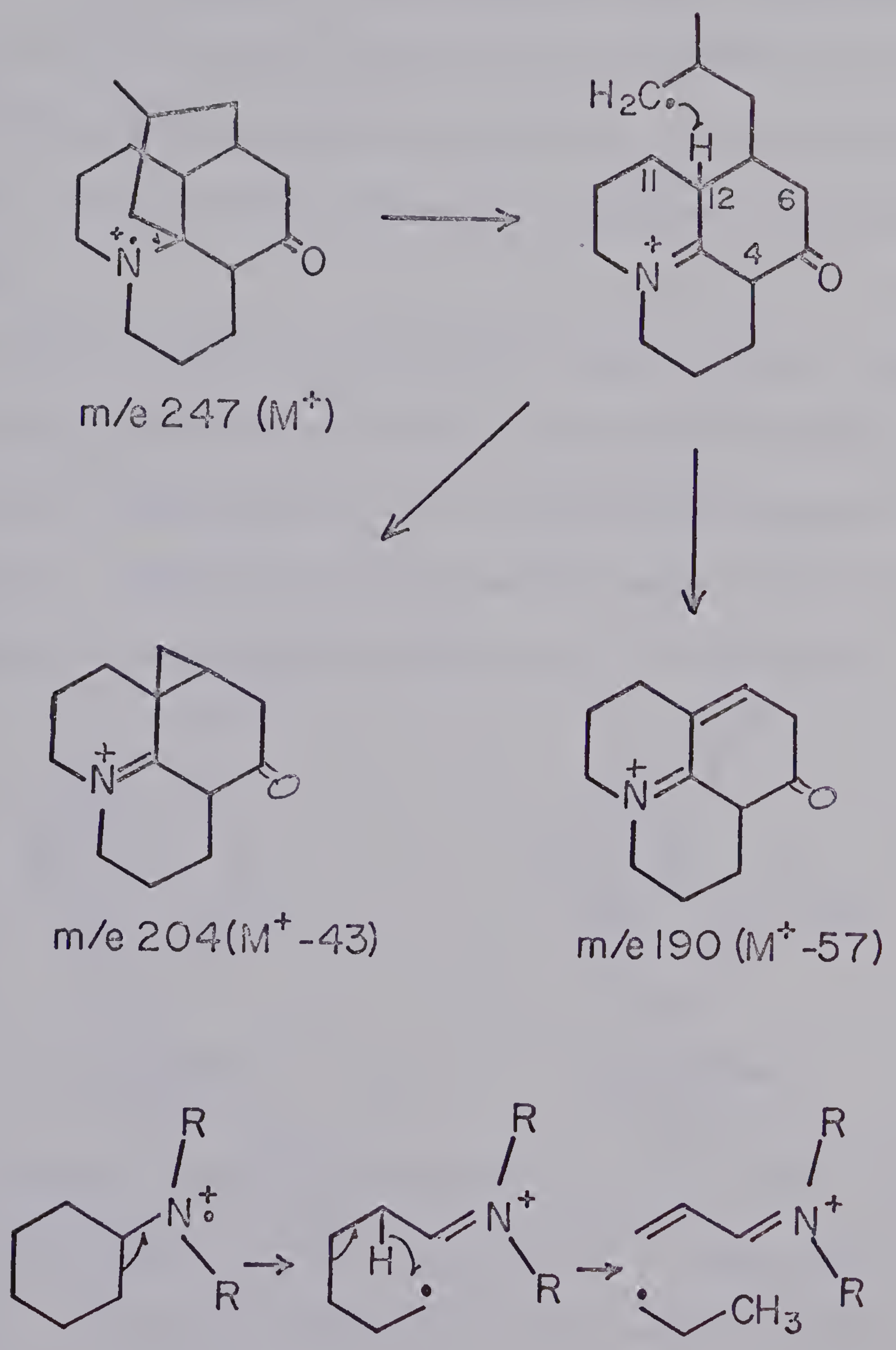


Lycodine[12]

Many of the advances achieved in the structural studies are due to new and improved methods of isolation as well as methods of structural analysis. Countercurrent distribution, thin-layer chromatography (tlc), elution chromatography and gas liquid partition chromatography (glpc) have all contributed greatly to the study of Lycopodium alkaloids. The elucidation of structure of the alkaloids has equally been aided by the development of physical methods. Nuclear magnetic resonance (nmr) has played a large part in this field of study (e.g., serratinine, Table I) as have infrared and ultraviolet spectroscopy. X-ray crystallography has both confirmed (e.g., luciduline, Table I) and established (e.g., annopodine, Table I) structures in this series of alkaloids. The application of mass spectrometry has been of great value in the elucidation of the structures of the Lycopodium alkaloids. MacLean[13] examined the spectra of a variety of alkaloids having the lycopodine skeleton. Since then mass spectrometry has been used in virtually all structural studies. A brief discussion of the spectra of some lycopodine type alkaloids is appropriate, since use has been made of this method in the work described in this thesis.

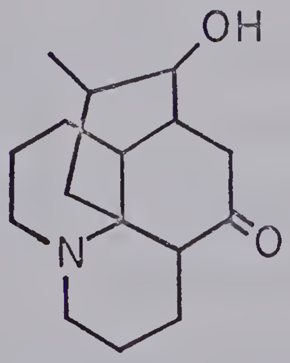
Upon electron impact lycopodine (2) shows prominent peaks at m/e 247 (M^+), 204 (M^+-43), 190 (M^+-57, base peak), 162(M^+-85) and 134(M^+-113) [15]. The fragmentation leading to the base peak involves the loss of the bridge as shown in Scheme I. It may be noted that this is a typical cyclohexylamine fragmentation route [14] also shown in Scheme I. The hydrogen lost with the bridge

Scheme I

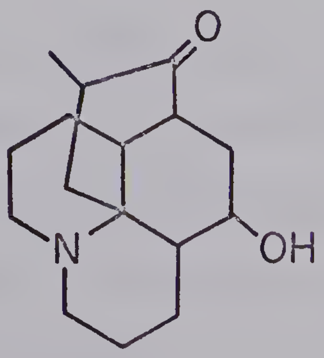

m/e 247 (M$^{+\cdot}$)
H$_2$C·
H
11
12
6
4
m/e 204(M$^+$-43)
m/e 190 (M$^{+\cdot}$-57)
R
N
CH$_3$

is postulated to come from position 12 since deuterium introduced at C-4 and C-6 is not lost and acrifoline, which has a double bond between C-11 and C-12, shows a very different fragmentation pattern[13]. The fragments showing *m/e* 162(M^+ -85) and *m/e* 134(M^+-113) represent consecutive losses of 28 units from the base peak (*m/e* 190) and are no doubt due to losses of carbon monoxide and ethylene. The order in which these units are lost has not been determined.

The presence of substituents on the bridge, as for example in clavolonine (3) and annofoline (4), does not alter appreciably the fragmentation pattern exhibited by lycopodine and its derivatives. Both the presence and the nature of the substituents on the bridge are immediately evident from an examination of the spectra. The base peak in the mass spectrum of clavolonine (3) is at *m/e* 190 indicating a loss of 73 mass units — bridge plus hydrogen plus oxygen. Likewise the base peak in the mass spectrum of annofoline at *m/e* 192 indicates the presence of a carbonyl on the bridge.



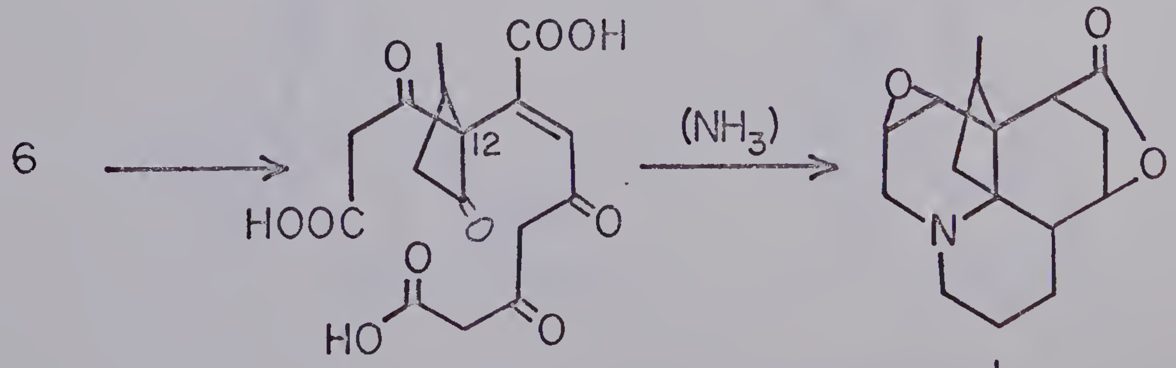
Clavolonine
3



Annofoline
4

Biogenetic considerations have played an important role in the structural elucidation of Lycopodium alkaloids, notably cernuine and lycocernuine[9,16], lycodoline[12] (5), annotine[17] and more recently fawcettidine[18], fawcettimine[19] and luciduline[10]. Conroy[20] proposed that the alkaloids might be formed from the condensation of two eight-carbon polyacetate chains. Thus the condensation of two 3,5,7-triketo-octanoic acid equivalents with one or more equivalents of ammonia accounts not only for the general construction of the alkaloids but also for many structural details. The sequence shown in Scheme II accounts for the biosynthesis of lycopodine (2) and lycodine (5). The polyacetate hypothesis of Conroy is the origin of the numbering system used for these alkaloids as an examination of Scheme II shows.

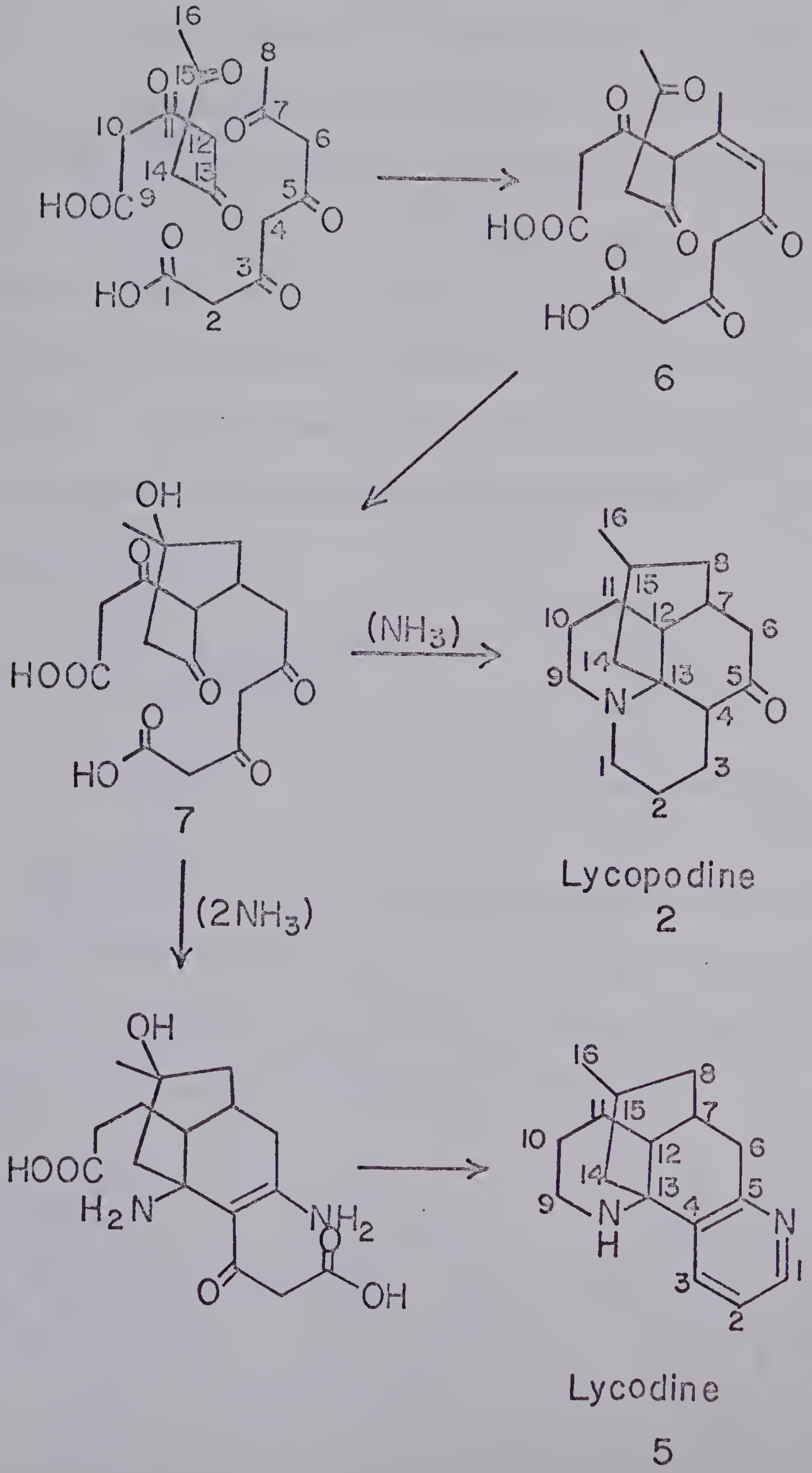
The route to annotinine (1) is a minor variant of the general pattern. In 6, Scheme II, if carbon 8 is oxidized to a carboxyl group before formation of 7, no aldol condensation can occur at C-8. An alternative ring closure at C-12 forming a cyclobutane ring could occur leading to annotinine (1).



1

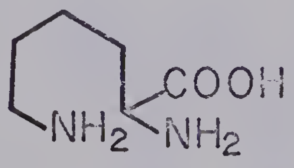
The hypothesis also accommodates the Lycopodium alkaloids having completely different skeletons. Two examples are

Scheme II


6
7
(NH3)
(2NH3)
Lycopodine
2
Lycodine
5

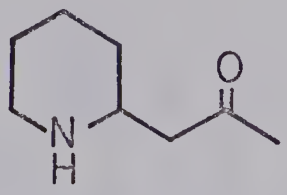
given in Scheme III.

More recently MacLean and coworkers[21] conducted experiments in which lysine-2-^{14}C and lysine-6-^{14}C were separately administered to *Lycopodium flabelliforme*. In each case radioactive lycopodine (2) was isolated. One fourth of the activity was found at the carbonyl carbon in each experiment. Further experiments led the authors to propose that lysine (8) serves as a precursor to lycopodine (2) via two pelletierine[22] units (9) as depicted in Scheme IV. The fact that lysine-6-^{14}C is specifically incorporated into the piperidine ring of N-methylpelletierine[23] lends further support to this hypothesis.



Lysine

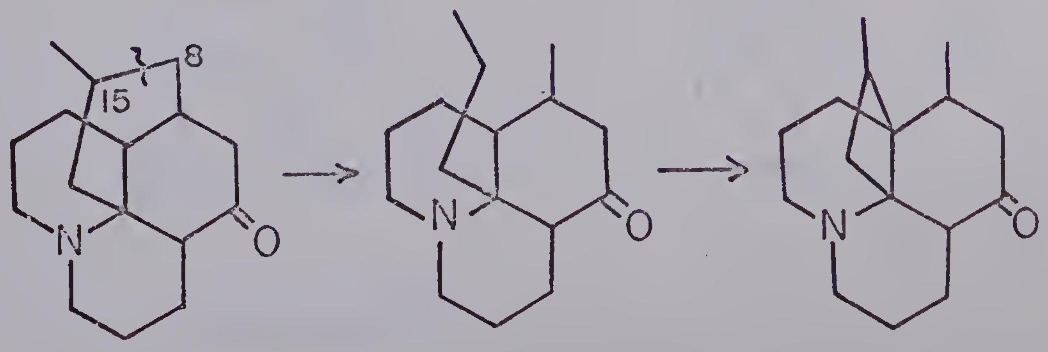
8



Pelletierine

9

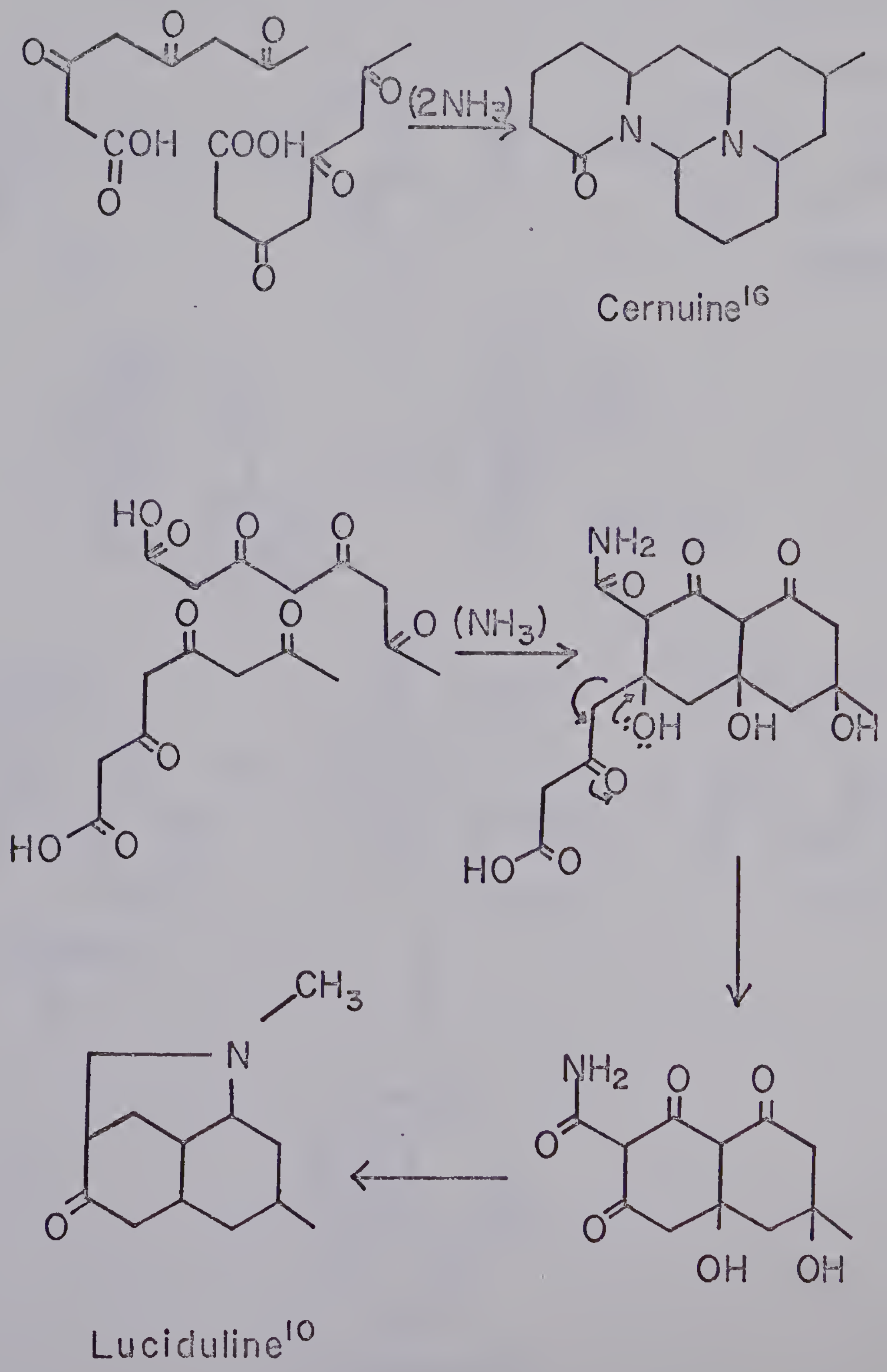
Annotinine (1) can be accommodated in this scheme if one considers that lycopodine (2) or some related form may be a precursor[24]. Cleavage of the C(8)-C(15) bond followed by recyclization could afford an annotinine system 10.



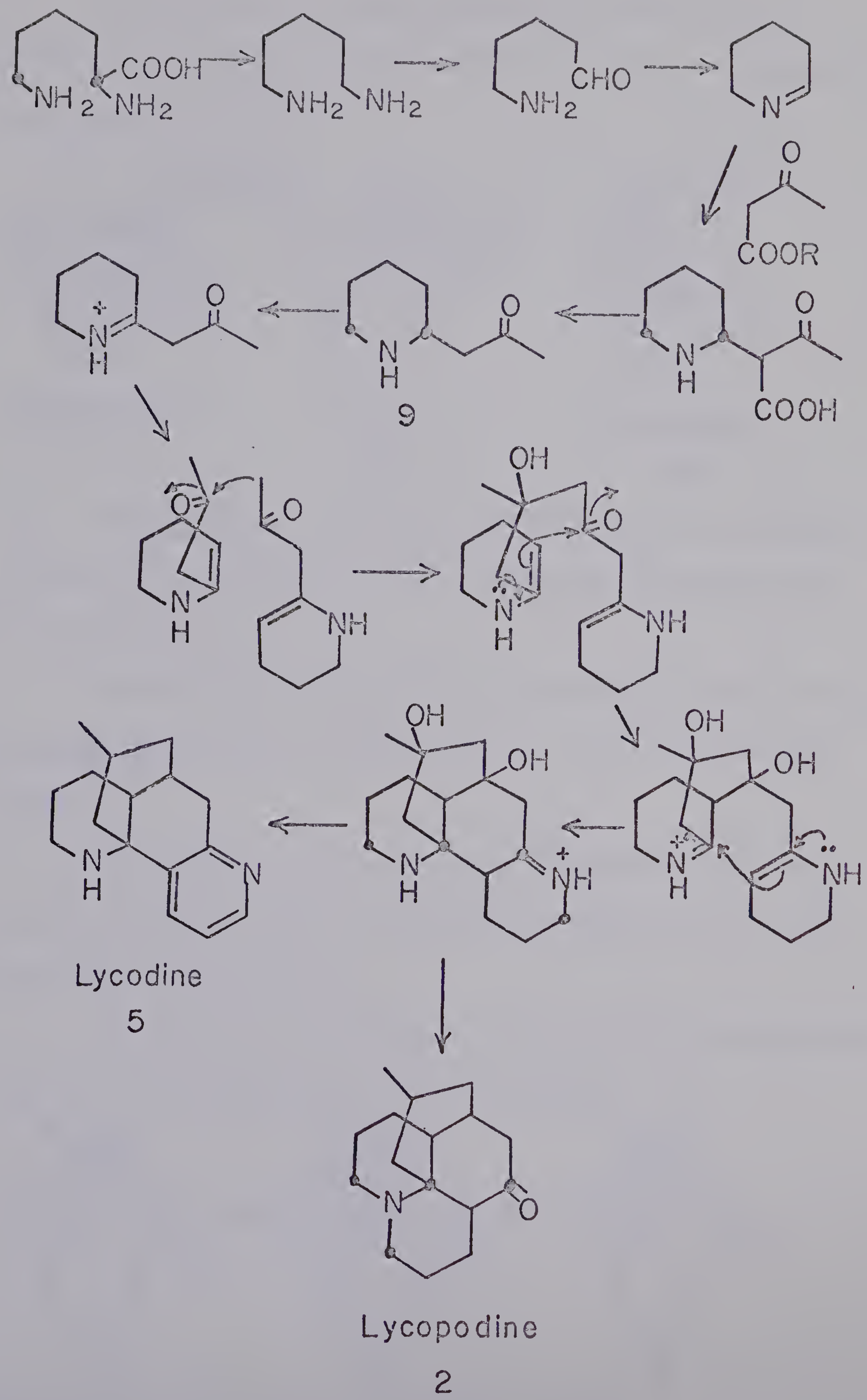
2

10

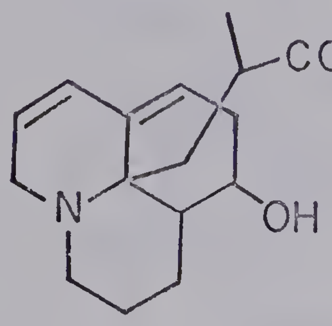
Scheme III


(2NH3)
Cernuine16
(NH3)
Luciduline10

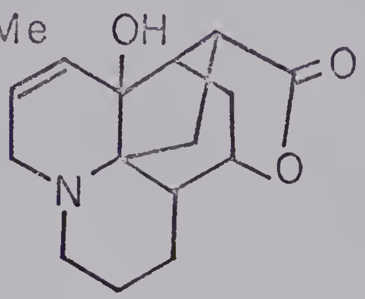
Scheme IV


COOH
NH2
NH2
NH2
NH2
CHO
NH2
N
O
COOR
+
N
H
O
N
H
O
9
N
H
O
COOH
O
O
N
H
NH
OH
O
N
H
NH
OH
OH
N
H
N
Lycodine
5
OH
OH
N
H
+
NH
OH
OH
+
N
H
NH
N
O
Lycopodine
2

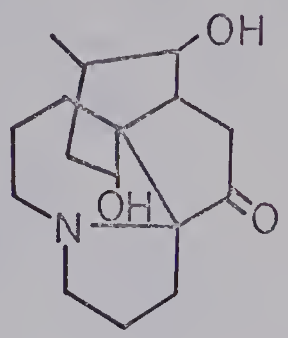
It has also been postulated that lyconnotine (11), annotine (12) and serratinine (13) could arise from rearrangement of the lycopodine skeleton [3].



Lyconnotine
11



Annotine
12



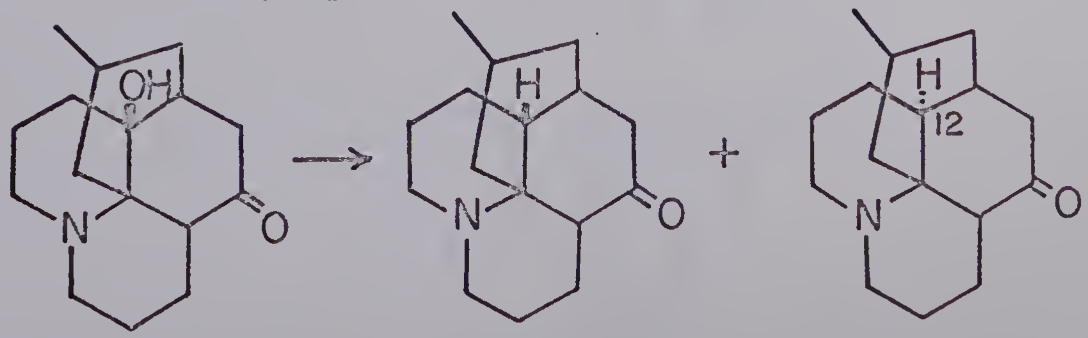
Serratinine
13

The biogenesis of luciduline and cernuine (see Scheme III) can easily be accommodated in the MacLean hypothesis as is shown in Scheme V.

In the course of elucidating the structure of the presently known Lycopodium alkaloids many of these were correlated with one another [3].

Lycopodine has been the main alkaloid for reference probably due to the fact that it is relatively plentiful and has been most extensively investigated.

Ayer and Iverach [25] were able to convert lycodoline (14) into a mixture of lycopodine (2) and epilycopodine* (15).

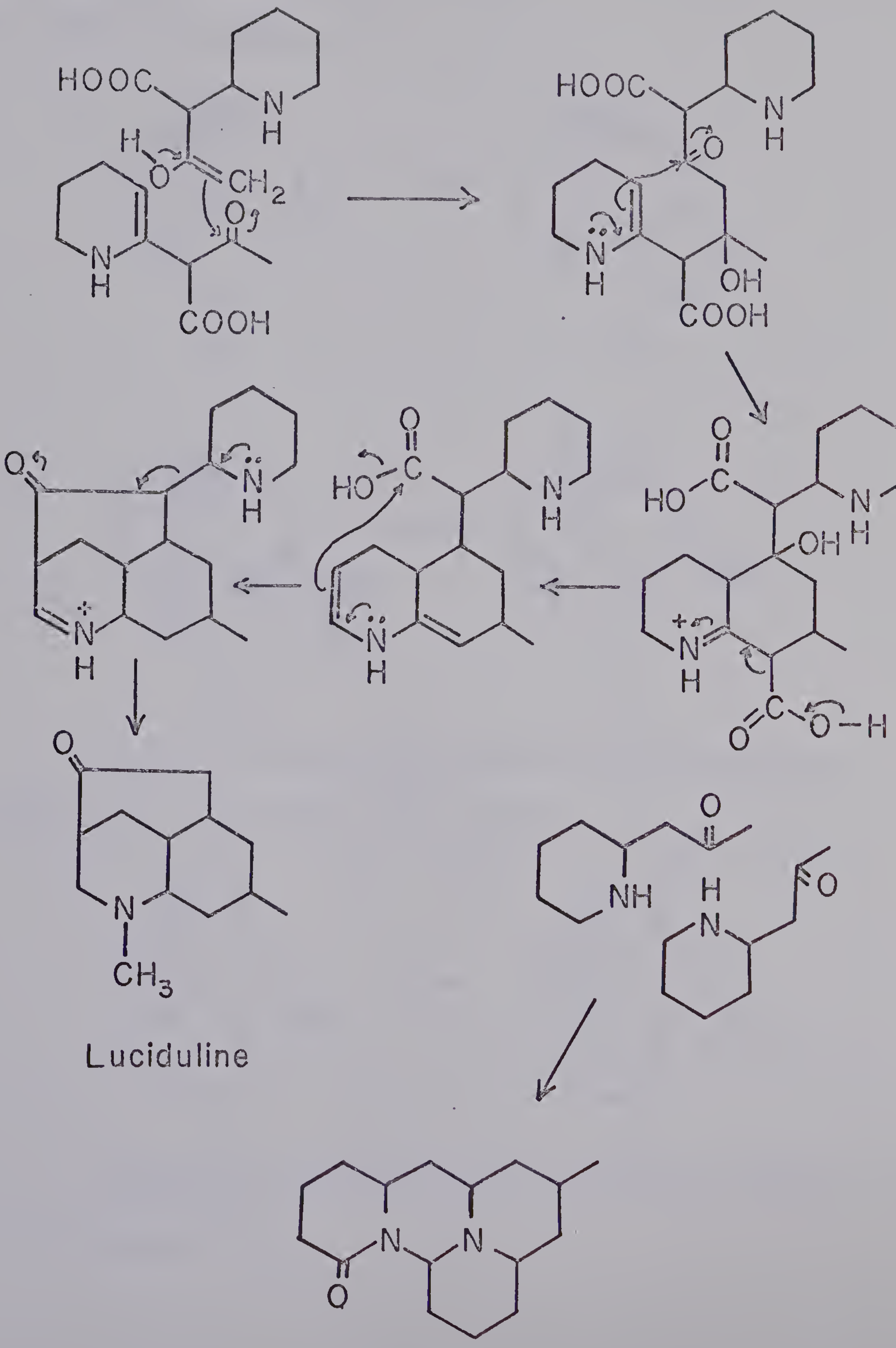


14 2 15

---

* Epilycopodine is the name of 12-*epi*-lycopodine.

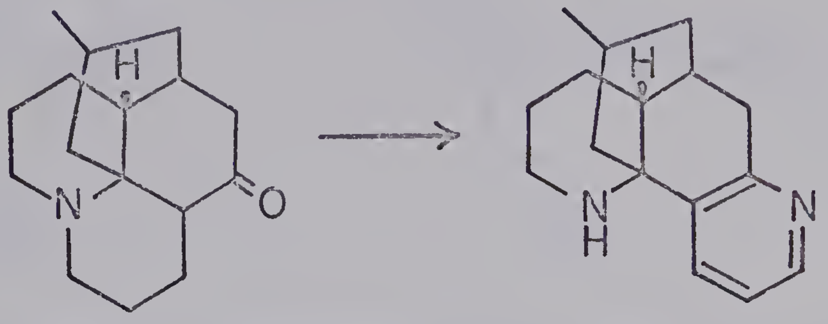
Scheme V


HOOC
NH
CH2
COOH
OH
HO
CH3
NH

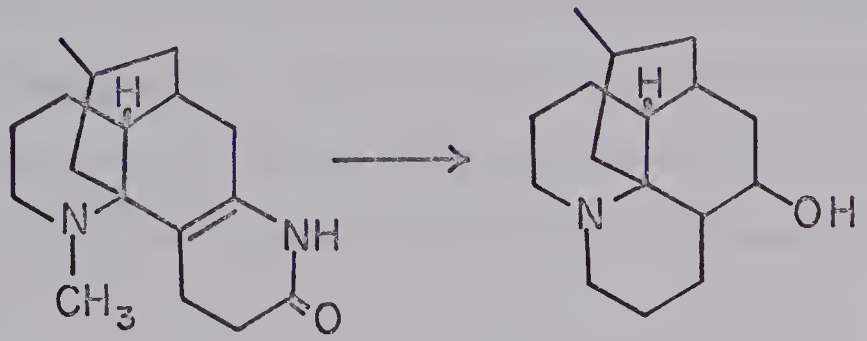
Luciduline

Cernuine

Anet and Rao[26] converted lycopodine (2) to lycodine (5) and at the same time Ayer and coworkers[27] converted α-obscurine (16) to dihydrolycopodine (17).

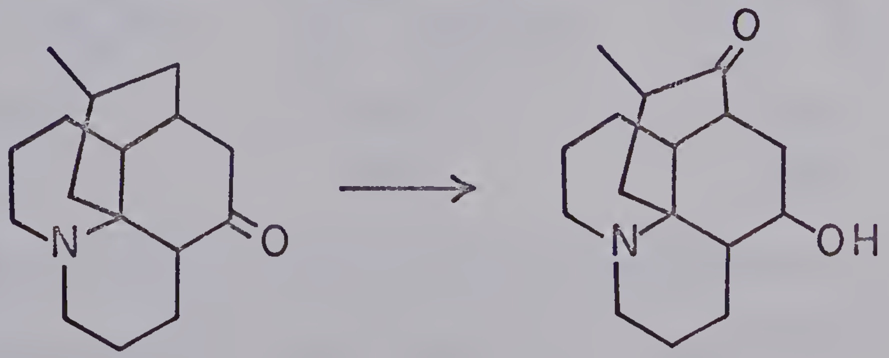


2 5



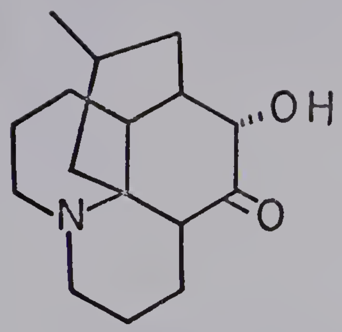
16 17

A few years later lycopodine (2) was converted to annofoline (4) by Ayer and coworkers[28].

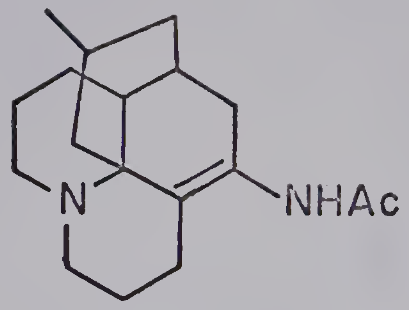


2 4

Lycopodine (2) has also been converted to alkaloid L.20[29] (18) and flabelline[30] (19).

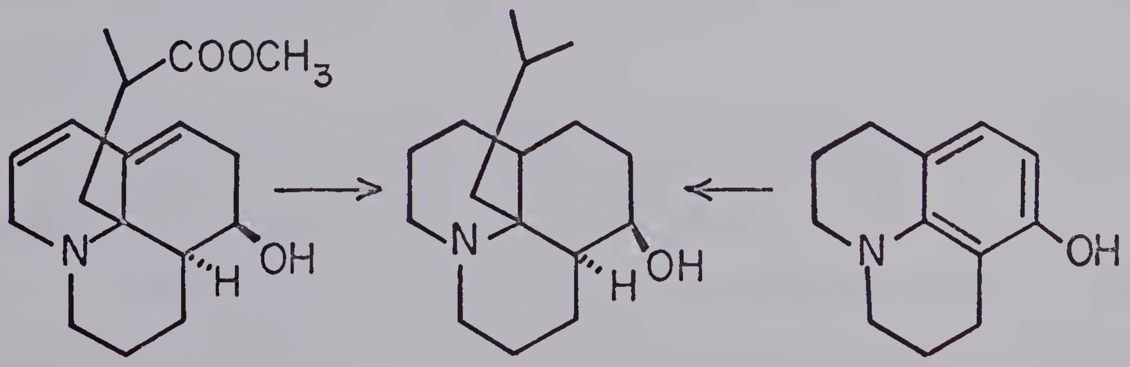


18



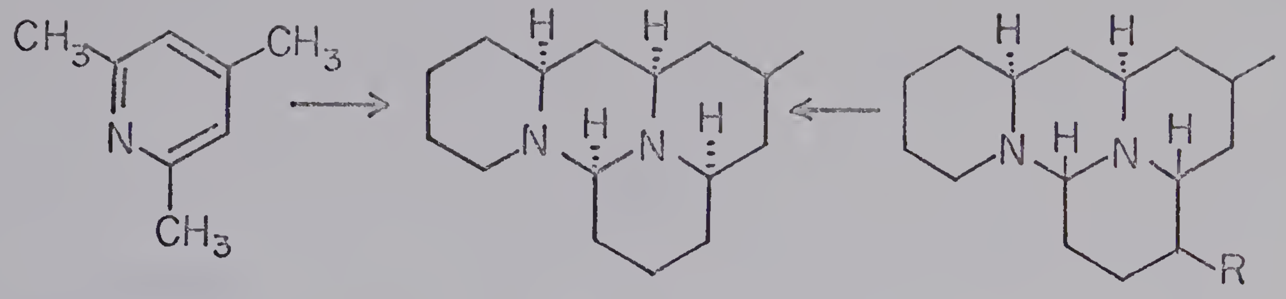
19

As well as making direct correlations between known alkaloids, some degradation products obtained in structural studies were independently synthesized. Wiesner, Valenta and coworkers[31] proved the structure of lyconnotine (11) by synthesizing one of its degradation products (20) from 8-hydroxyjulolidine (21).



11 20 21

In the course of the structural elucidation of cernuine (22) and lycocernuine (23), Ayer and Piers[32] synthesized dihydrodeoxyepiallocernuine (24) which had been obtained from lycocernuine[9, 33] (23).



24

22 R = H

23 R = OH

Since the first report of an attempted synthesis of lycopodine (2) in 1964 by Bohlmann and coworkers[34] there have been many other attempts reported[25, 36, 37].

The first successful synthesis of lycopodine (2) was simultaneously reported in 1968 by Stork and coworkers[38] and by Ayer and coworkers[39]. These two methods for the preparation of lycopodine (2) also represent in a formal sense the synthesis of many of the other Lycopodium alkaloids which have been synthesized from lycopodine.

In 1967 the research group at the University of New Brunswick reported a synthesis of annotinine (1)[40].

The alkaloids having different skeletons from lycopodine (2) and annotinine (1) now represent interesting synthetic problems.

The work presented in this thesis can be divided into two main parts. In the first part the alkaloids of L. alopecuroides L. are investigated. From this plant six new alkaloids were obtained and the structure of each examined.

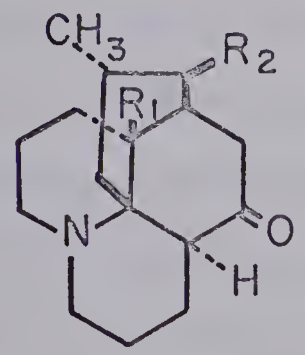
An examination of the alkaloids from the strong bases of L. lucidulum constitutes the second part of this thesis.

DISCUSSION AND RESULTS

PART I

1. Isolation

The presence of alkaloids in *L. alopecuroides* L. has been noted previously[41,42]. Monroe et al[41], in an extensive screening program of the native flora of the United States of America, reported a positive Mayer alkaloid test for *L. alopecuroides*. More recently Valverde[42] isolated six compounds from this lycopod by chromatographic separation of the crude alkaloid extract adsorbed on alumina. These compounds included lycopodine (2), lycodoline (14) and clavolonine (3) of known structure and three that had not been reported in the literature previously. These three new alkaloids were given the names alopecurine, alopecuridine and "base 16" but no structures were assigned at that time.



2 $R_1 = H$, $R_2 = H$ lycopodine
14 $R_1 = OH$, $R_2 = H$ lycodoline
3 $R_1 = H$, $R_2 = OH$ clavolonine

The information then available on these alkaloids suggested that they did not contain the tetracyclic-lycopodine skeleton and that further investigation of these structures would be justified. In particular, it appeared that alopecurine contained a pentacyclic skeleton, a structural type not previously encountered in this group

of alkaloids.

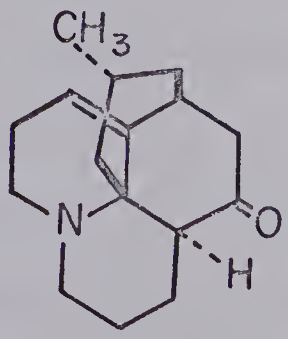
Alopecuridine, $C_{16}H_{25}NO_3$, has properties similar to fawcettimine, $C_{16}H_{25}NO_2$, (vide infra), an alkaloid isolated from L. Fawcettii and it seemed probable that alopecuridine was an hydroxylated fawcettimine. The complete structure of fawcettimine was not known at the time. "Base 16" was assigned the molecular formula $C_{16}H_{25}NO$ on the basis of mass spectral data. Since "base 16" is resistant to hydrogenation and shows no absorption in the carbonyl region of the infrared spectrum, it too appeared to be pentacyclic in structure.

In the present investigation a total of ten alkaloids were isolated and characterized. These included five of the compounds isolated by Valverde[42], however, no "base 16" could be found even though a thorough search was conducted.

The separation of the alkaloids of L. alopecuroides was achieved by a combination of chromatographic techniques. Adsorption on alumina of the crude alkaloid extract followed by elution with solvents of increasing polarity effected a preliminary separation. The progress of the separation was monitored by thin-layer chromatography. The fractions thus obtained were further separated by gas liquid partition chromatography, "dry-column" chromatography[43] and by crystallization. The technique of "dry-column" chromatography is based on the fact that, as in thin-layer chromatography, development is effected by movement of an eluent over dry absorbent using only the solvent absorbed by capillary action. Thus, a column

is dry-packed and developed by allowing the solvent to move down mainly by capillary action (aided by the pull of gravity) using a solvent head of only 1-2 cm. When the solvent reaches the bottom, development is complete and the components are isolated by extrusion and slicing or by further elution. In order to facilitate the presentation, the alkaloids will be discussed in order of decreasing R_f value.

The total crude alkaloid mixture was obtained from Smith, Kline and French Laboratories, Philadelphia, who used the isolation procedure reported by Ayer _et al_[44]. A portion of this material was chromatographed over basic alumina. Elution with benzene furnished an oil which contained three components. "Dry-column" chromatography was successful in separating two of the components in pure form. One of these, an unstable oil, appeared from the mass spectrum to be anhydrolycodoline[25] (25). This was confirmed by an examination of the crystalline hydrochloride salt.



25

The natural occurrence of anhydrolycodoline had not been noted previously. The other component to be separated by "dry-column" chromatography was lycopodine (2), the most widely distributed of the _Lycopodium_ alkaloids[45]. The third component of the mixture

was separated in pure form by gas liquid partition chromatography (glpc). The first component to emerge from glpc column was anhydrolycodoline which had already been characterized. The second compound to emerge from the glpc column was also an oil, moderately stable in air, that was isolated as a crystalline picrate salt. The melting point and infrared spectrum of this picrate were identical with those of fawcettidine picrate*. Although fawcettidine has a larger R_f than anhydrolycodoline on tlc it has a longer retention time on glpc. The elucidation of the structure of fawcettidine will be described in Section 2.

Elution with ether yielded a mixture of two alkaloids of similar R_f value. Crystallization from acetone afforded one of the components in pure state. This component, the structure and properties of which will be described in Section 4, has been named alopecurine. Concentration of the mother liquors from this crystallization furnished crystals of lycodoline [25], the identity of which was established by comparison with an authentic sample. The next component eluted with ether was acetyldebenzoylalopecurine. It was apparent from the infrared, nuclear magnetic resonance and mass spectra of this alkaloid that the benzoyl group of alopecurine had been replaced by an acetyl group. Confirmation of this was obtained by a chemical correlation of the two alkaloids as described in Section 4.

---

* Kindly supplied by Dr. R. H. Burnell.

Elution with chloroform furnished clavolonine[46] (3) identified by its melting point and infrared spectrum. "Dry-column" chromatography of the mother liquors of this crystallization furnished an oil, homogeneous by tlc, that displayed a characteristic band in the infrared spectrum at 1640 cm^{-1}. After unsuccessful attempts to crystallize this oil it was treated with acetic anhydride in pyridine solution and gave a crystalline O-acetyl derivative. Hydrolysis of the purified O-acetyl derivative produced the original compound which crystallized from ether solution and was given the name alolycopine. Subsequently, the infrared spectra of all the mother liquors were taken and those displaying a 1640 cm^{-1} band were combined and acetylated. The O-acetylalolycopine was easily separated, purified and hydrolyzed to give crystalline alolycopine. The structure of the O-acetylalolycopine was deduced from the ultraviolet, infrared, nuclear magnetic resonance and mass spectra as described in Section 3. The final chemical proof was obtained when the hydrogenation of O-acetylalolycopine produced acetylanhydroaposerratinine[8], a compound of known structure.

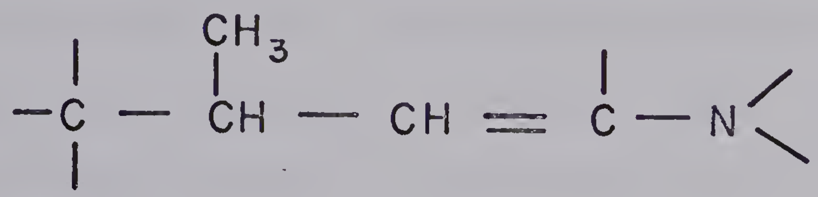
Further elution provided another new alkaloid which has been given the name alopecuridine. The interesting properties of this alkaloid are related to a plausible structure in Section 2. Elution with methanol-chloroform yielded a small amount of debenzoylalopecurine. Whether this alkaloid occurs in the plant or is formed during the isolation procedure by hydrolysis of alopecurine is not known.

2. (a) The Structure of Fawcettidine

Fawcettidine (Base F) was first isolated by R. H. Burnell[47] from a Jamaican plant, L. fawcettii Lloyd and Underwood, by distillation of the crude basic extract. The preparation of the picrate and the methiodide was reported. A sample of dihydrofawcettidine (45 mg), prepared by sodium borohydride reduction of fawcettidine, was supplied by Burnell and used in the present investigation. Oxidation of the dihydrofawcettidine with Jones' reagent furnished fawcettidine which displays characteristic bands in the infrared spectrum (CCl_4) at 1740, 1660 and 1415 cm^{-1}. The 1740 cm^{-1} band disappeared on reduction with sodium borohydride and was assigned to a cyclopentanone carbonyl. The 1415 cm^{-1} band indicated the presence of a methylene group adjacent to the carbonyl ($-C\underline{H_2}CO-$). This band is absent from the spectrum of dihydrofawcettidine. The 1660 cm^{-1} band was assigned to a double bond and this was confirmed by the presence of one olefinic proton in the nuclear magnetic resonance spectrum of fawcettidine. Because of the relatively high intensity of this 1660 cm^{-1} band it was thought that it might be adjacent to the nitrogen. The proximity of the double bond to the nitrogen would also account for the low basicity (pK_a 6.4)[47] of fawcettidine compared to lycopodine and the shift to lower field of the olefinic proton in the nuclear magnetic resonance spectrum of fawcettidine in perdeutero acetic acid (τ 3.37) compared to the chemical shift in deuterochloroform (τ 4.1). The nuclear magnetic resonance spectrum of fawcettidine shows a methyl signal

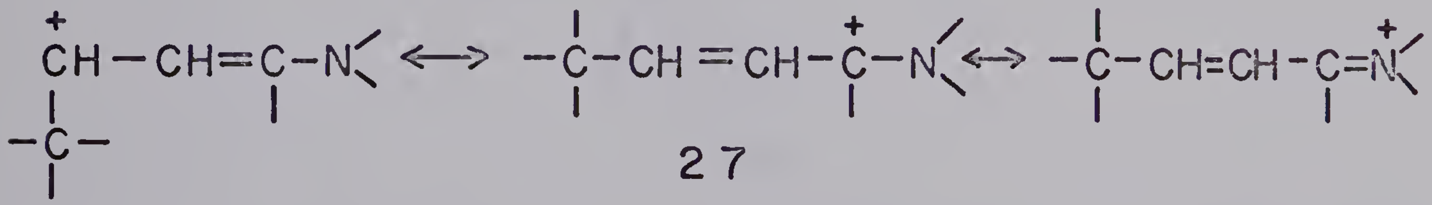
at τ 8.86 (3H, d, J=7 cps). The relationship between this methyl group and the olefinic proton (τ4.1, 1H, d, J=5 cps) was established by spin decoupling experiments. Double irradiation at τ 7.68 spin decouples both the olefinic doublet and the methyl doublet.

This gave a part structure (26) in which the methyl group is allylic to a vinylamine. A structure such as this was consistent

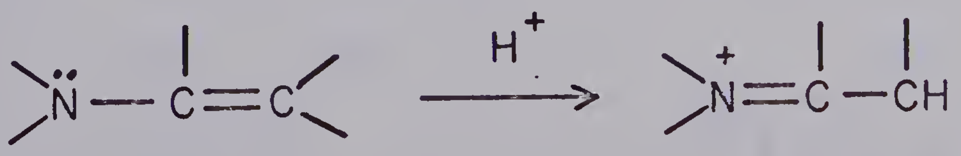


26

with the mass spectrum of fawcettidine (figure 7a, page 40) which shows a strong M^+-15 (*m/e* 230) indicating a facile loss of methyl radical to give a resonance stabilized ion (27).



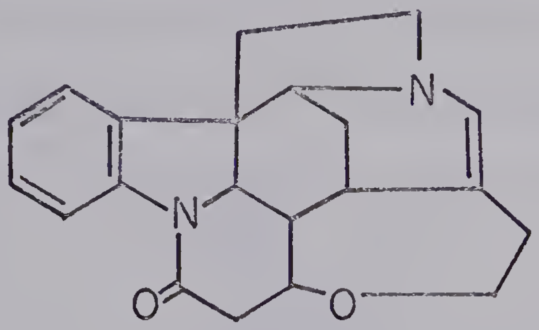
Vinylamines usually show a hypsochromic shift of double bond stretching frequency in the infrared (20-50 cm^{-1}) on salt formation[48] as shown in the sequence 28 → 29.



28 29

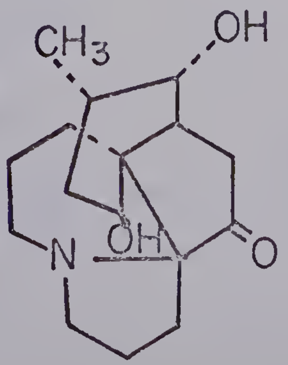
The position of the double bond stretching frequency in the infrared spectrum of O-acetyldihydrofawcettidine (1660 cm^{-1}) remained unchanged on hydroperchlorate formation. Also, the

chemical shift of an enamine [49] proton is in the region of τ5.5 while the olefinic proton in dihydrofawcettidine is at τ4.47. Both of the above results can be accommodated in the part structure 26 if a cage structure is postulated in which no overlap occurs between the p orbitals of the double bond and the orbital containing the lone electron pair on nitrogen. In this case, N-, rather than C-protonation occurs on salt formation. Neostrychnine (30) is an example of such a vinylamine that does not show a shift of double bond stretching frequency in the infrared on salt formation due to a bridged structure [48].



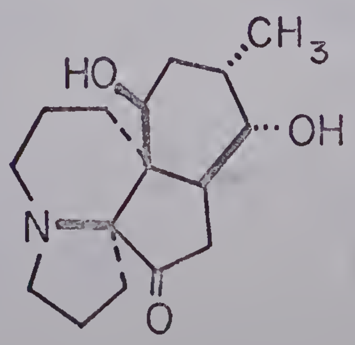
30

A review of the structures of the lycopodium alkaloids known at that time, showed that only one contained a cyclopentanone group. This was serratinine (13), the major alkaloid from L. serratum Thunbergii [7]. An inspection of the structure showed that in no



13

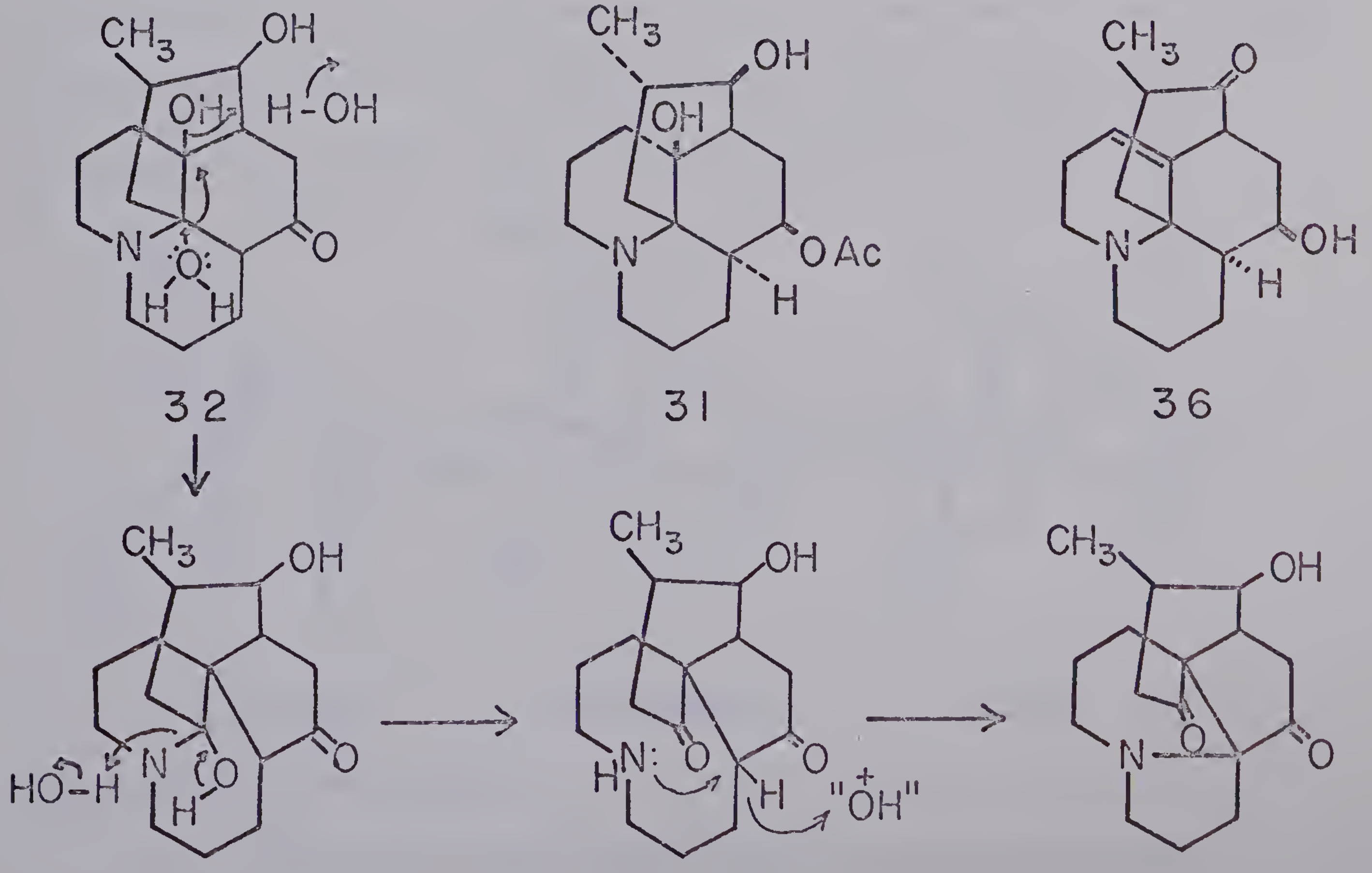
≡



13a

simple way can part structure 26 be incorporated into the skeleton of serratinine (13). It was thus concluded that fawcettidine had a skeleton unique among lycopodium alkaloids.

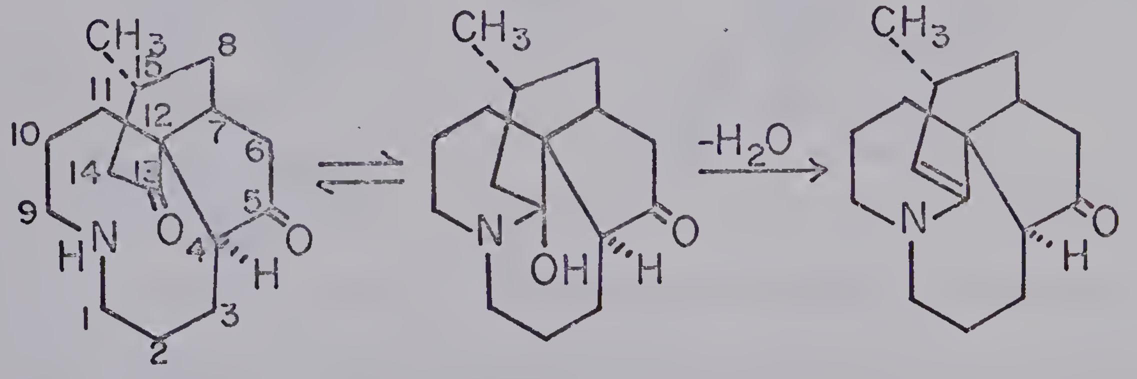
Since serratinine (13) was at that time (spring of 1966) the only known lycopodium alkaloid with a cyclopentanone ring, it seemed possible that fawcettidine might be biogenetically related to serratinine. Inubushi and coworkers[50] speculated that the serratinine ring system might arise by rearrangment of an alkaloid with the lycopodine skeleton. They suggested that a precursor such as 32, with the oxygenation pattern found in lycofawcine[51] (31), might undergo rearrangment to the serratinine system in the manner depicted in the sequence 32 → 35. Acrifoline (36) could serve equally well as a precursor for rearrangment to the serratinine system.



32 31 36

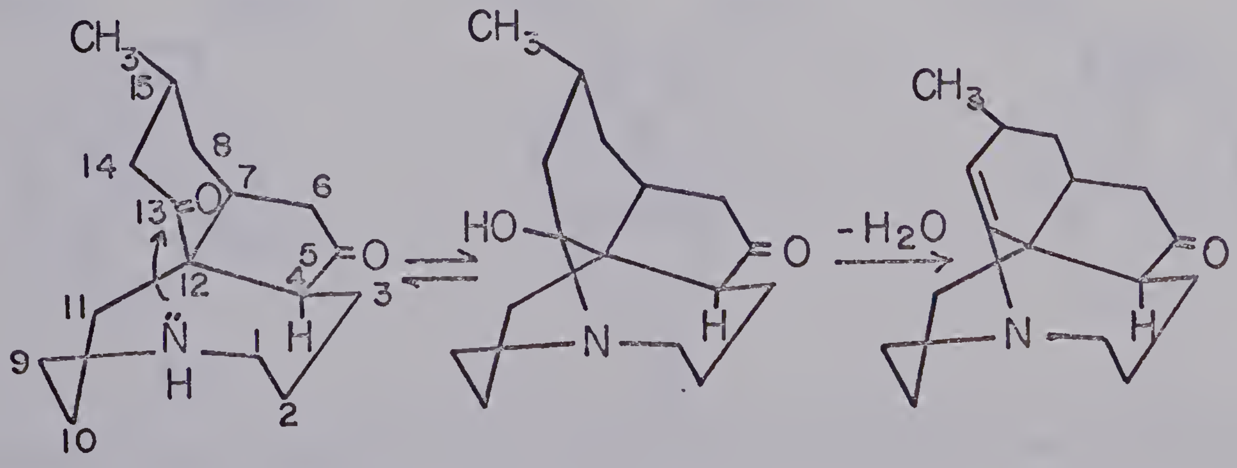
33 34 35

At that time it was felt (see Appendix 1, copy of letter to Prof. Y. Inubushi) that fawcettimine, an alkaloid isolated from L. fawcettii by Burnell [47] might be related to the hypothetical intermediate 34 of this biogenetic scheme and be represented by structure 37 (34, OH replaced by H). It was conceivable that dehydration of 38, the carbinolamine form of 37 would give a compound, 39, which would possess the properties of fawcettidine.


CH3
15
8
11
12
10
7
6
14
13
5
9
N
H
O
4
H
O
1
3
2
CH3
N
OH
H
O
-H2O
CH3
N
O
H

37 38 39

In particular, 39 is an enamine in which the lone pair on the nitrogen cannot overlap effectively with the double bond as shown in the perspective drawing 39a and would be expected to display all the

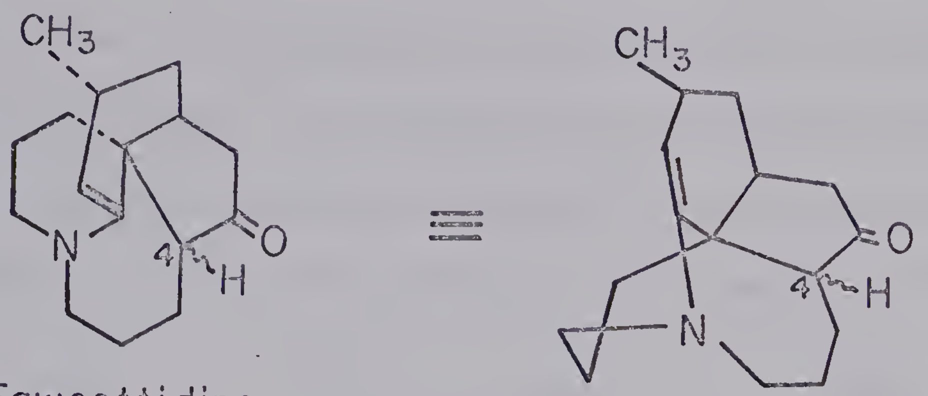

CH3
15
14
8
7
6
13
O
5
O
12
4
3
11
N
H
H
1
9
2
10
CH3
HO
O
N
H
-H2O
CH3
O
N
H

37a (=37) 38a (=38) 39a

properties of fawcettidine.

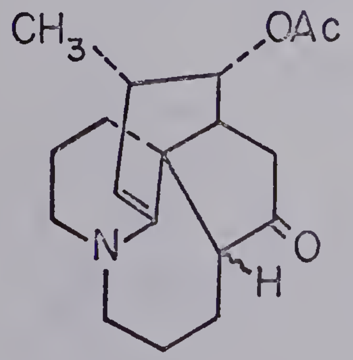
At the time when structure 39 was seriously being

considered as a likely structure for fawcettidine, but had not yet been proven, Inubushi and coworkers[18] published a structure for fawcettidine (40), the same as 39, except the stereochemistry at C-4 was unspecified. The weak basicity of fawcettidine, the presence of a double bond in the molecule and the infrared band at 1740 cm^{-1} together with the hypothetical biogenesis of serratinine type alkaloids led the authors to suppose that the skeletal structure of fawcettidine was the same as that of acetylanhydroaposerratinine (pK$_a$ 6.4) (41). Acetylanhydroaposerratinine had been obtained by Inubushi and coworkers[8] in an attempt to transform serratinine (13) into lycodoline (14). The purpose of this transformation was to lend support to the suggested biogenetic scheme. Serratinine (13) was monoacetylated in cold pyridine-acetic anhydride solution.

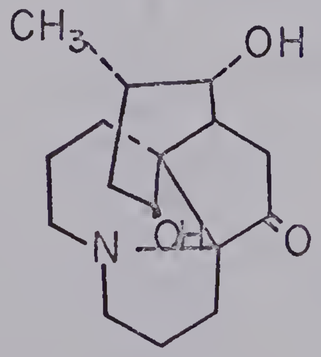


Fawcettidine

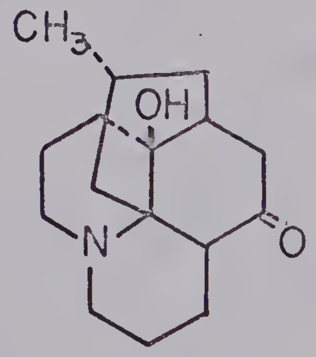
40



41

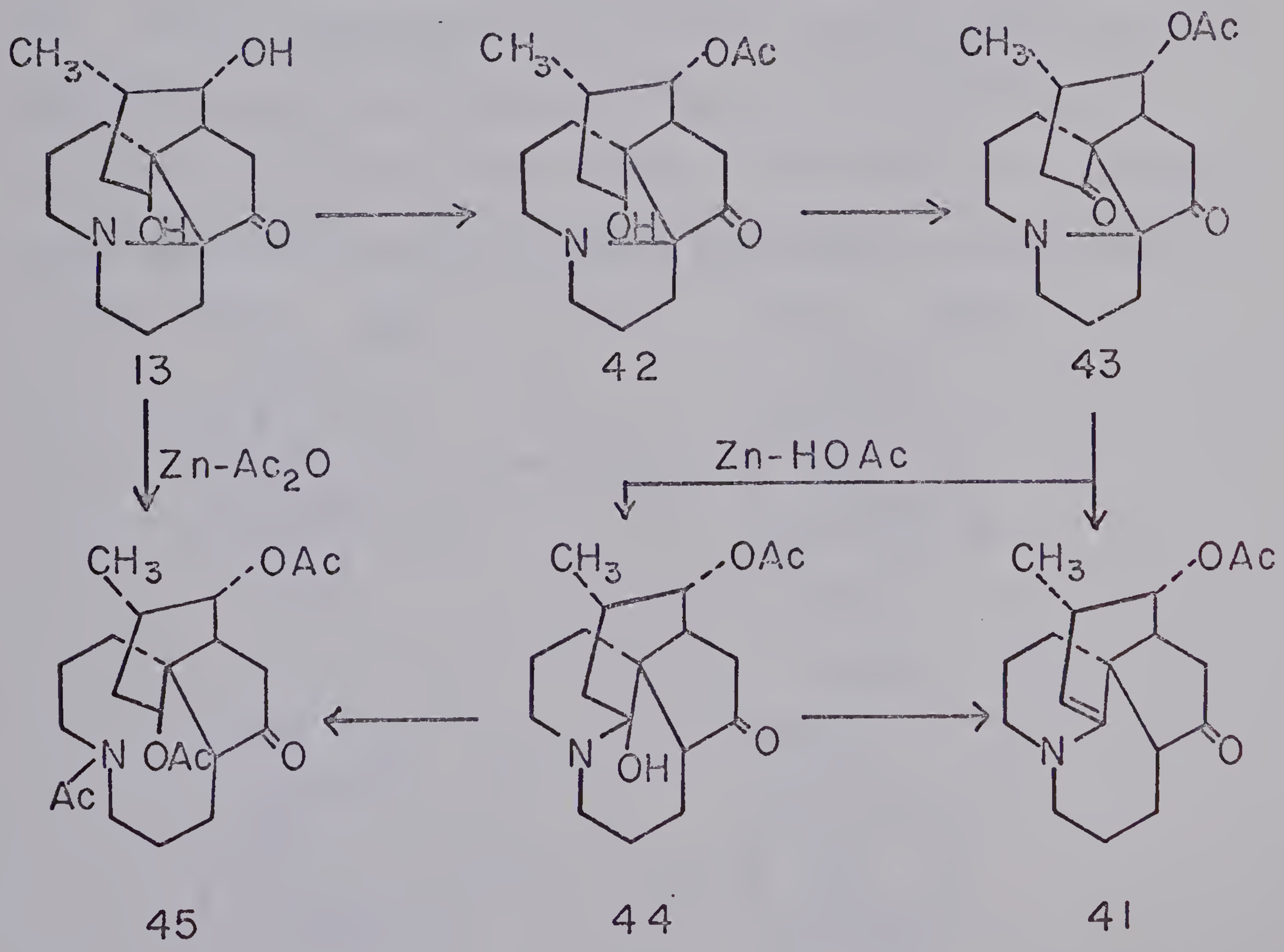


13



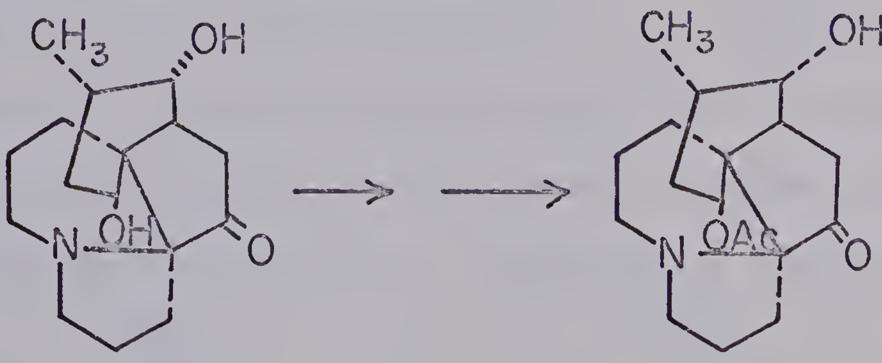
14

The monoacetylserratinine I (42) thus obtained was oxidized by Jones' reagent to dehydromonoacetylserratinine I (43). Compound 43 was reduced with zinc in acetic acid and yielded acetylanhydroaposerratinine (41) and acetylaposerratinine (44). Compound 44 was transformed into compound 41 by dehydration with phosphorus oxychloride in pyridine. Compound 44 was also related to O,O,N-triacetylchanodihydroserratinine (45), the compound obtained from serratinine (13) by reduction with zinc in acetic anhydride.

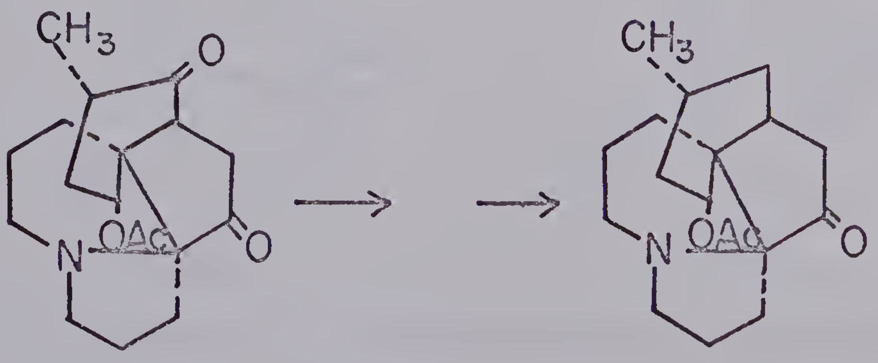

CH3
OH
N
OH
O
13
CH3
OAc
N
OH
O
42
CH3
OAc
N
O
O
43
Zn-Ac2O
Zn-HOAc
CH3
OAc
N
Ac
OAc
O
45
CH3
OAc
N
OH
O
44
CH3
OAc
N
O
41

Inubushi and coworkers established the structure of fawcettidine by the synthesis of 40. In the synthesis of 40 they applied the reduction process that had been used to transform

serratinine (13) into acetylanhydroaposerratinine (41) and chose 13-dehydro-8-deoxyserratinine (46) as the starting material in view of the lack of an hydroxyl group in fawcettidine. In order to prepare compound 46, the authors first converted serratinine (13) into the diacetyl derivative by refluxing in pyridine solution with acetic anhydride. Selective hydrolysis was achieved by refluxing in aqueous hydrochloric acid (10%) to give monoacetylserratinine II (47) which on Jones' oxidation gave dehydromonoacetylserratinine II (48). Raney nickel desulfurization of the monoethylenethioketal of dehydromonoacetylserratinine II (48) gave 13-acetyl-8-deoxyserratinine (49), a key compound in the elucidation of the structures of both fawcettidine and fawcettimine (vide infra). Hydrolysis of



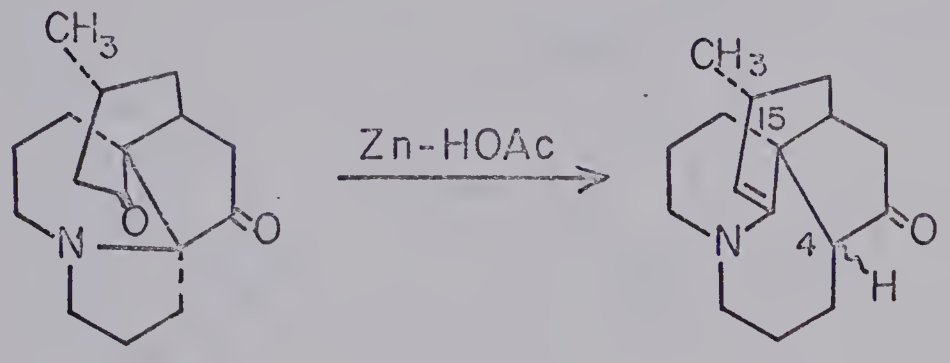
13 47



48 49

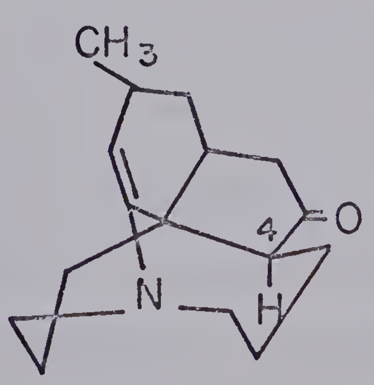
49, followed by oxidation with Jones' reagent, furnished 13-dehydro-8-deoxyserratinine (46), which on reduction with zinc in acetic acid

afforded an oil, the physical constants of which agreed with those of fawcettidine (40) reported by Burnell[47, 52]. This result established the structure of fawcettidine except for the stereochemistry at C-15 and C-4. The stereochemistry of the methyl group in fawcettidine was assigned the same configuration as in lycodoline[25, 53] (14) from the biosynthetic considerations already discussed.

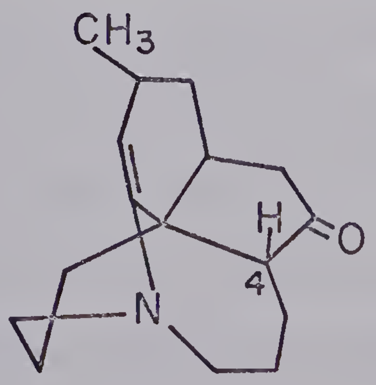


46 40

The stereochemistry at C-4 in fawcettidine (40) can be either as shown in structure 39a or structure 50a. Since this centre is adjacent to a carbonyl group epimerization can occur and fawcettidine, which is not isomerized by acid or base, will exist in the thermodynamically more stable form. Inspection of molecular models



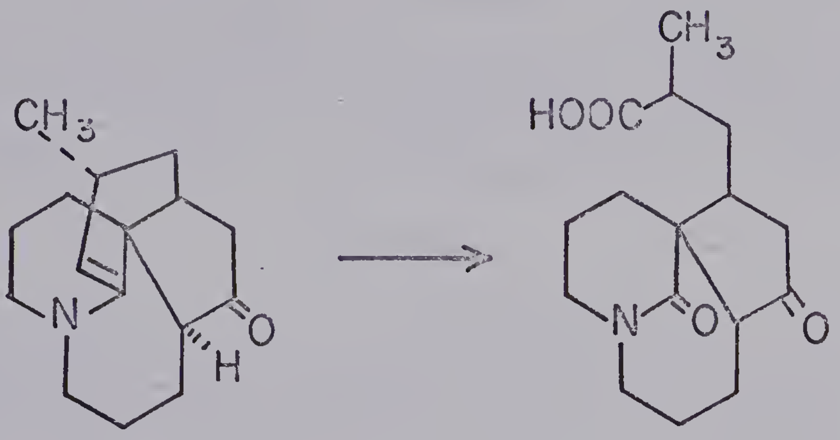
39a



50a

clearly shows that structure 50a has severe non-bonded interactions not present in 39a and therefore it is reasonable to postulate that 39a represents the structure and stereochemistry of fawcettidine.

With the appearance of the paper by Inubushi and coworkers[18] the structural studies on fawcettidine in these laboratories were discontinued. Preliminary experiments to establish the structure by oxidative cleavage of the carbon-carbon double bond, (39 → 51), had been made but had not been successful.



39 51

2. (b) The Structure of Fawcettimine

As was mentioned in Section 2(a), structure 38 appeared, on biogenetic grounds, to be a reasonable working hypothesis for the structure of fawcettimine.

Fawcettimine (Base A) was first isolated in 1959 from *L. fawcettii* Lloyd and Underwood as its crystalline perchlorate[47] and formulated as $C_{16}H_{27}NO_2$. The formula was later[52] revised to $C_{16}H_{25}NO_2$. Fawcettimine showed carbonyl absorption in the infrared at 1732 cm^{-1} as well as hydroxyl absorption at 3585 cm^{-1} (figure 1). Acetylation of fawcettimine furnished a neutral N-acetyl derivative[54] which showed no OH or NH absorption in the infrared spectrum but which now showed carbonyl absorption at 1730, 1690 and 1615 (N-COCH$_3$) cm^{-1} (figure 2). These properties of

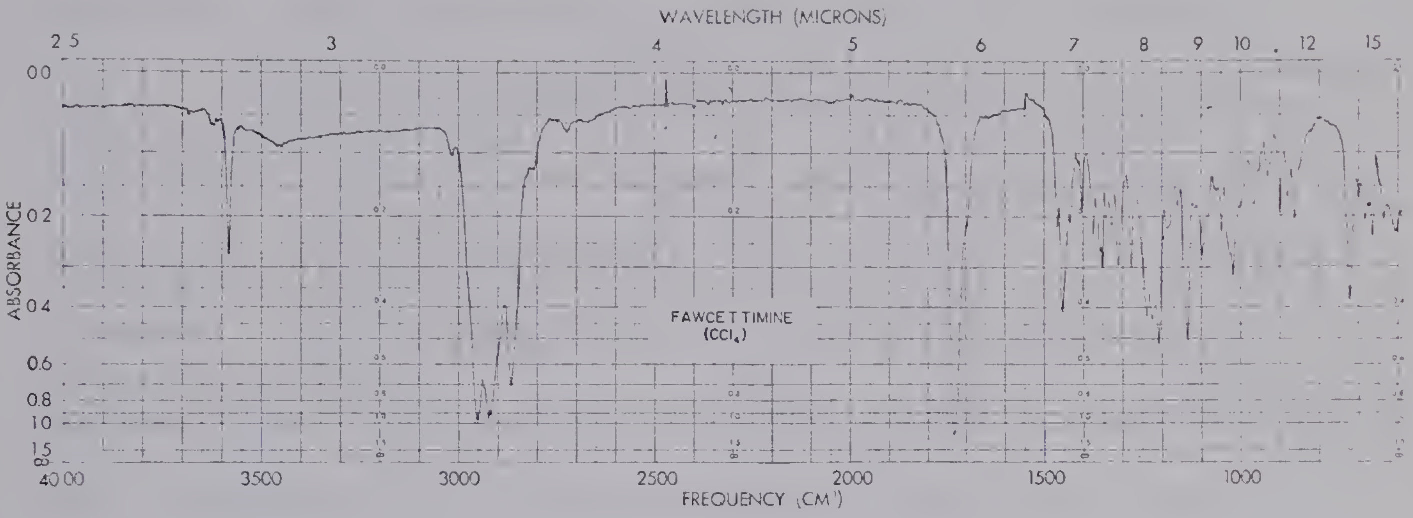


Figure 1

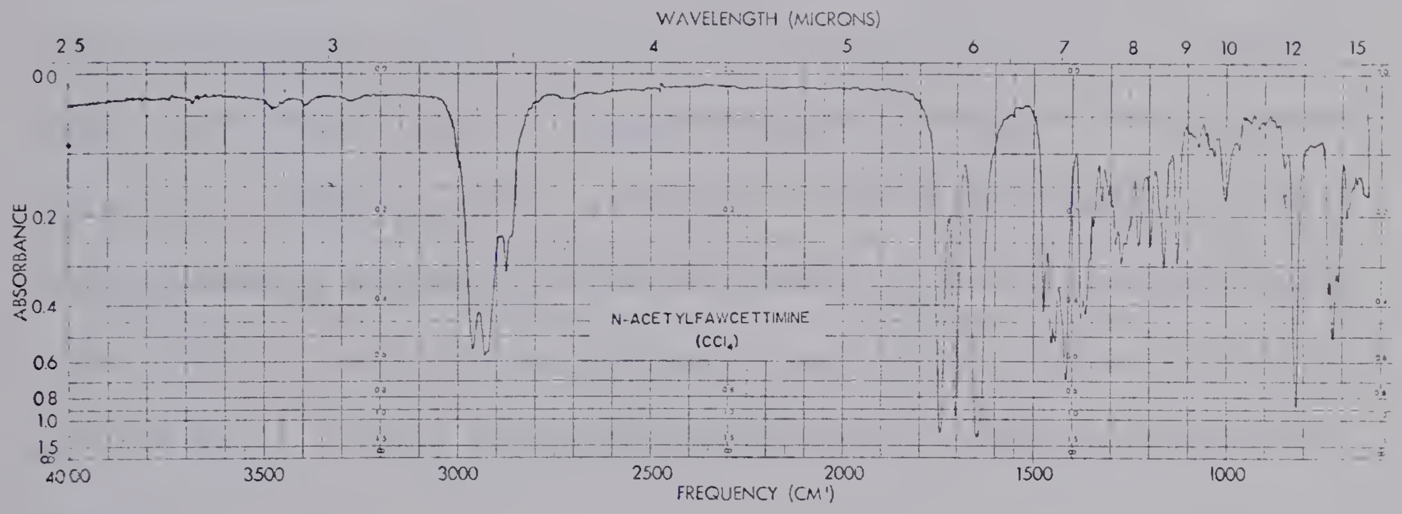
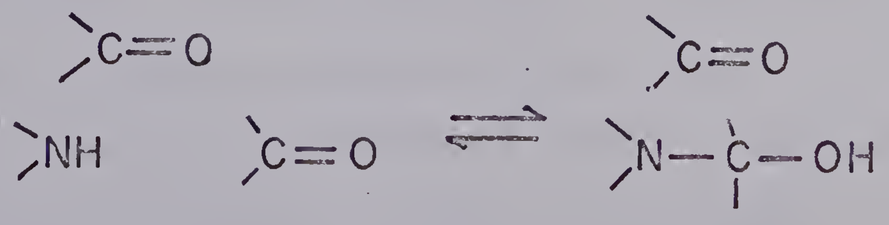


Figure 2

fawcettimine were rationalized in terms of a transannular interaction[55] between a secondary amino group and a carbonyl group of fawcettimine as summarized in part structures 52 and 53.

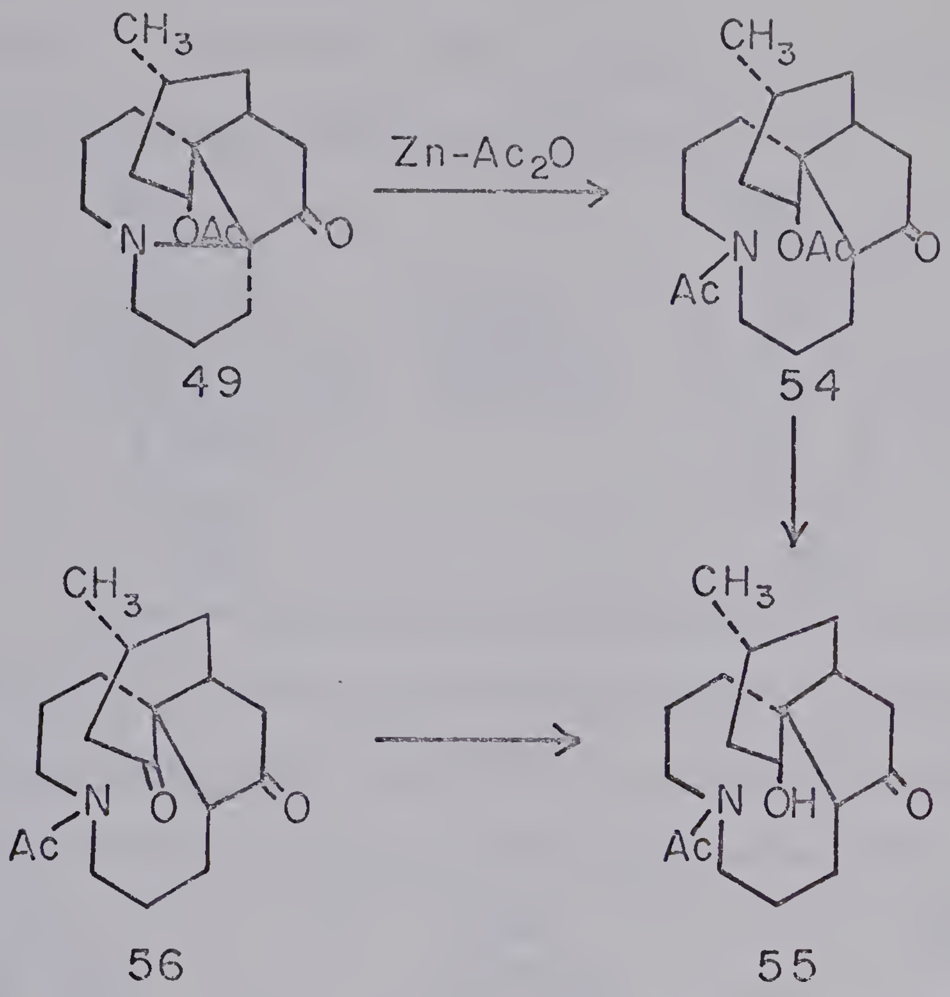


52 53

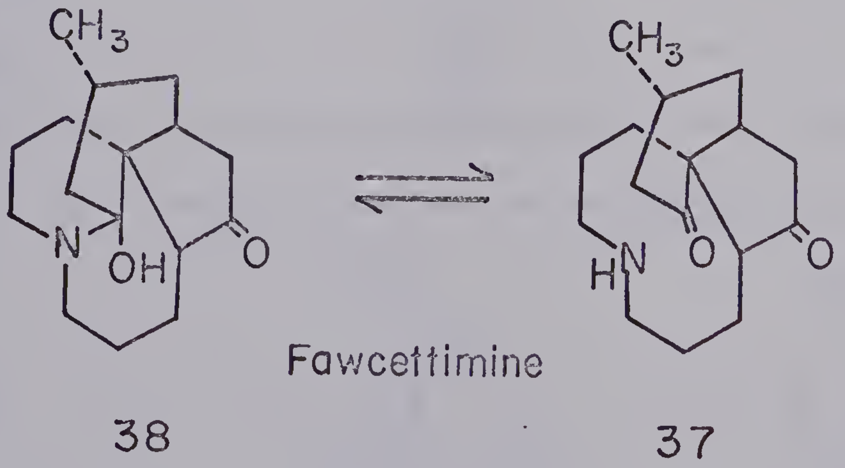
In solution fawcettimine exists predominantly in form 53, while the N-acetyl derivative is of form 52. The fact that the interacting

carbonyl group is a ketonic carbonyl rather than an aldehydic carbonyl is indicated by the lack of absorption for an aldehydic proton in the nuclear magnetic resonance spectrum of N-acetylfawcettimine. The nuclear magnetic resonance spectrum of fawcettimine reveals the presence of a secondary C-methyl group at $\tau 8.93$. Lack of olefinic absorption in the nuclear magnetic resonance spectra of fawcettimine and N-acetylfawcettimine and their resistance to hydrogenation dictates against the presence of an olefinic linkage. The fact that the absorption band due to the non-interacting carbonyl group in fawcettimine and its derivatives absorbs at 1730-1740 cm^{-1} suggested that it was a cyclopentanone carbonyl. Reduction of N-acetylfawcettimine with either sodium borohydride or sodium-alcohol furnished dihydro-N-acetylfawcettimine (55)[54], which shows hydroxyl, amide and carbonyl absorption at 1735 cm^{-1}. This indicated that it retained the five-membered ketone.

On the basis of the suggestion by Ayer and coworkers (see Appendix 1, copy of letter from Prof. Y. Inubushi) Inubushi and coworkers[18] attempted a direct correlation between serratinine (13) and fawcettimine. Such a correlation was achieved by the reduction of 13-acetyl-8-deoxyserratinine (49) with zinc in acetic anhydride to give N,O-diacetylchanodihydro-8-deoxyserratinine (54) which on hydrolysis gave dihydro-N-acetylfawcettimine (55).


CH3
N
OAc
O
Zn-Ac2O
Ac
49
54
56
55
OH

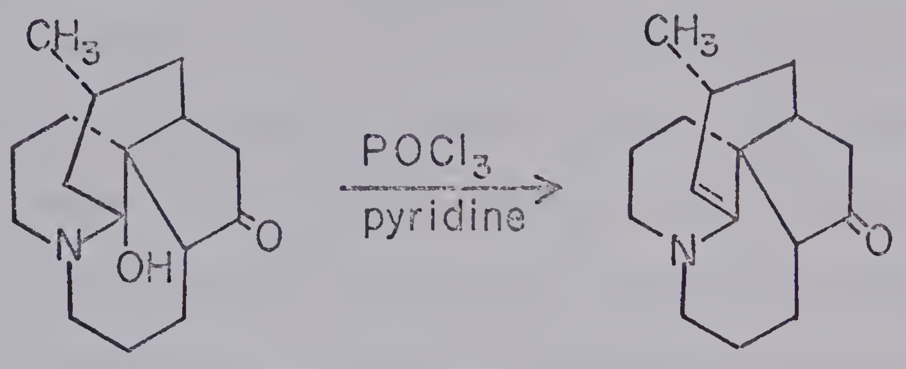
The above result indicates that fawcettimine has structure 38, that can exist in a carbinolamine (38) or an amino-keto (37) form.


CH3
N
OH
O
HN
Fawcettimine
38
37

N-acetylfawcettimine has structure 56 which on reduction gives dihydro-N-acetylfawcettimine (55).

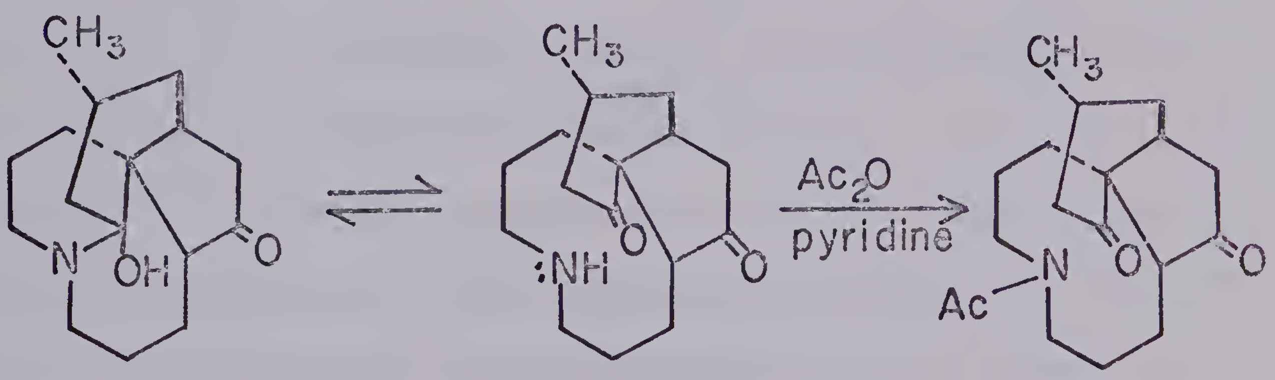
Confirmation of our earlier hypothesis that fawcettidine could be formed by the dehydration of fawcettimine was obtained by

experiment. Dehydration of fawcettimine with phosphorus oxychloride in pyridine solution gave fawcettidine (39).

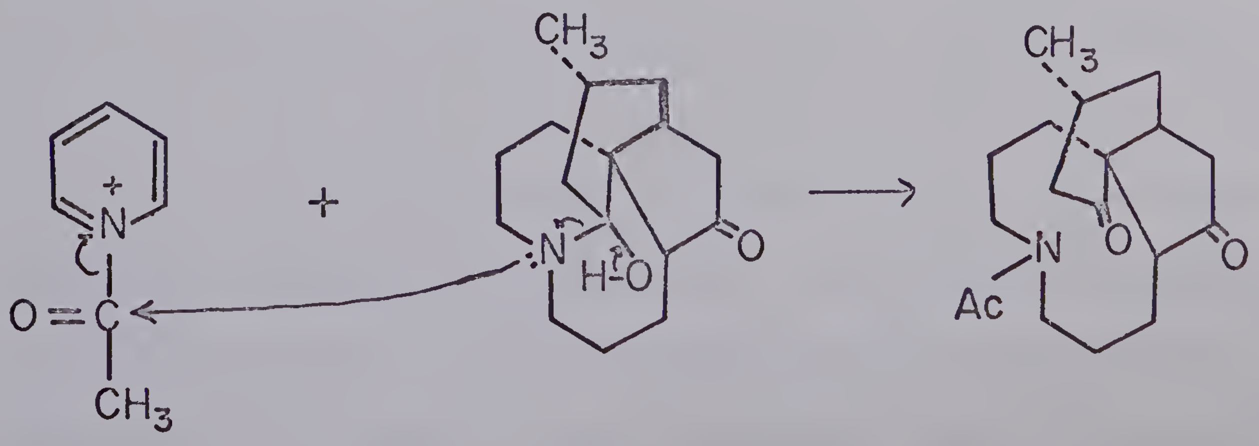

CH3
OH
O
N
POCl3
pyridine
CH3
N
O

38 39

The mechanism for the acetylation of fawcettimine (38) is not known. It could have occurred in either or both of two ways. Acetylation of the amino-keto form 37 would lead directly to N-acetylfawcettimine (56). Acylation could also have occurred by electrophilic attack at the nitrogen of the more predominant carbinolamine form 38. Besides being more nucleophilic than oxygen, the


CH3
N
OH
O
CH3
:NH
O
O
Ac2O
pyridine
CH3
N
Ac
O
O

38 37 56


+
N
O=C
CH3
+
CH3
N
H-O
O
CH3
N
Ac
O
O

57 38 56

nitrogen is less hindered to attack by the acylating species 57.

2. (c) The Structure of Alopecuridine

Analytical and mass spectral data indicated the molecular formula $C_{16}H_{25}NO_3$ for alopecuridine. The infrared spectra of this substance was reminiscent of that of fawcettimine. The spectrum of alopecuridine determined in chloroform (figure 3) shows strong OH absorption at 3575 cm^{-1} and weak absorption at 3330 cm^{-1} (NH?). In the carbonyl region there is strong absorption at 1740 cm^{-1}, medium intensity absorption at 1690 cm^{-1} and weak absorption at 1595 cm^{-1}. The nujol mull spectrum of alopecuridine, which had been crystallized from acetone, (figure 4) exhibits a series of bands in the OH, NH region, as well as carbonyl absorption at 1730 (strong) and 1640 cm^{-1} (medium intensity). The nujol mull spectrum of a sublimed sample of alopecuridine (figure 5) shows a sharp band at 3470 cm^{-1} and a single carbonyl band at 1710 cm^{-1}. Both of these crystalline forms exhibit the solution spectrum mentioned above. The infrared spectra of the hydroperchlorate and the hydrobromide (Nujol) show a single carbonyl band at 1750 cm^{-1}. Acetylation of alopecuridine yields a neutral N-acetyl derivative, $C_{18}H_{27}NO_4$, which shows hydroxyl absorption, as well as carbonyl absorption at about 1740, 1685 and 1620 cm^{-1} (both in solution and Nujol mull) (figure 6). As in the case of fawcettimine these results could best be interpreted on the basis of a transannular interaction between a secondary amino group and a carbonyl group of alopecuridine.

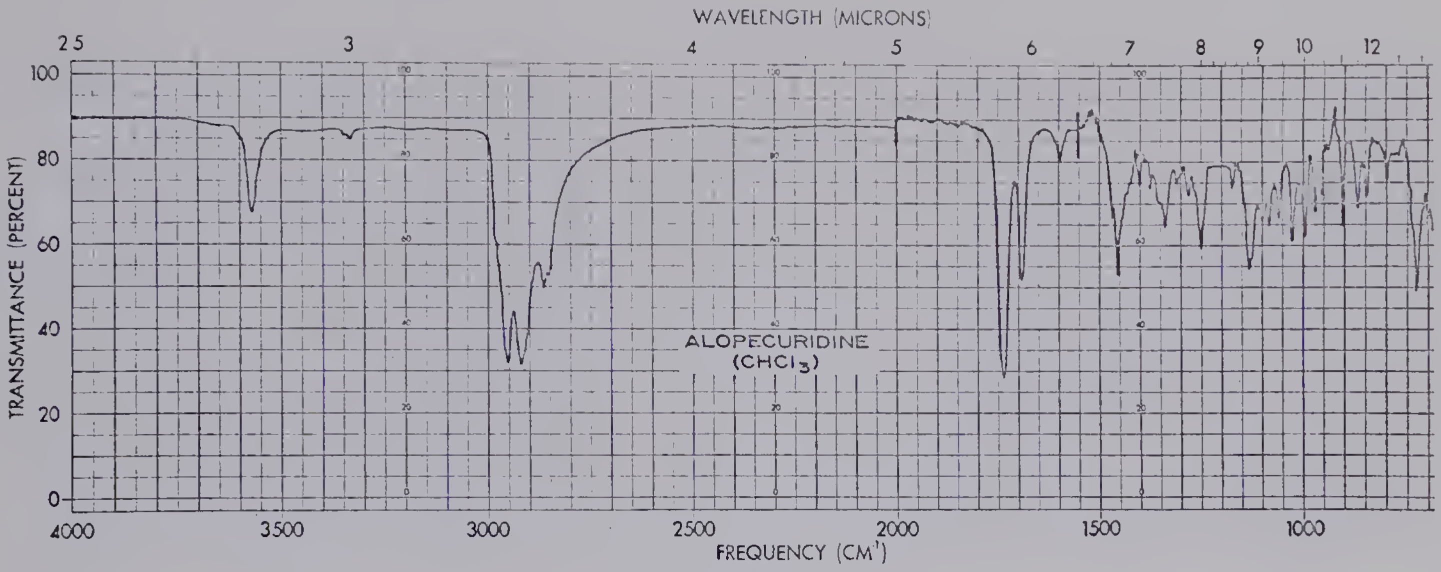


Figure 3

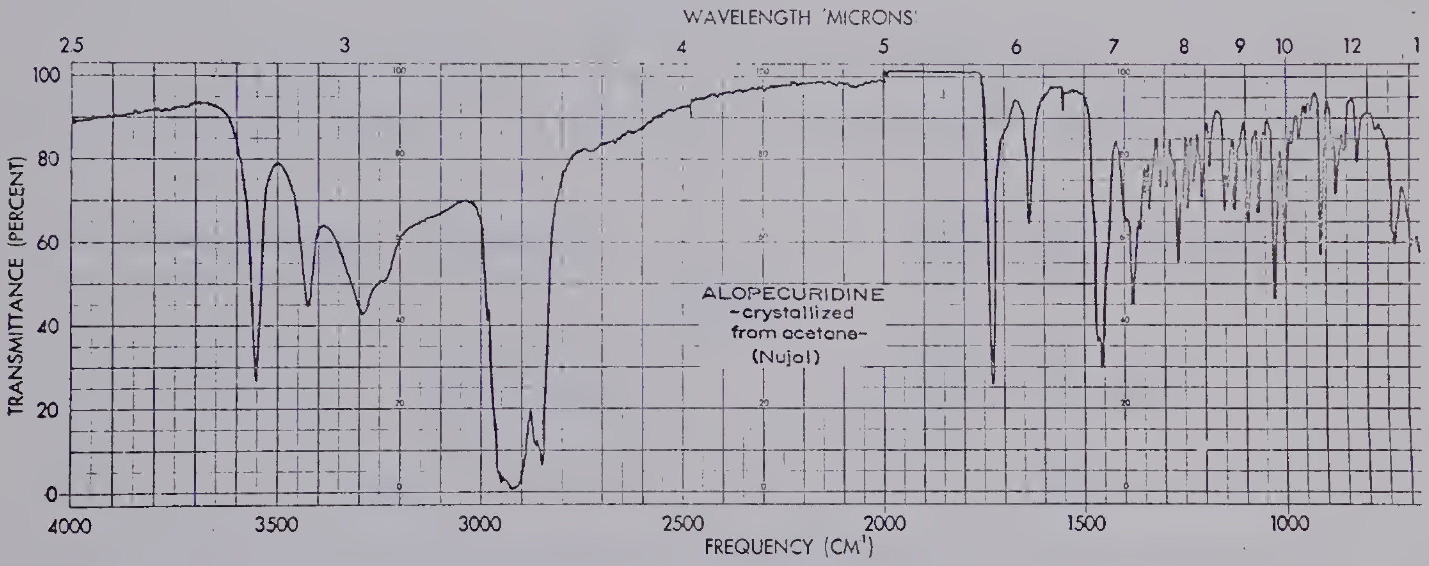


Figure 4

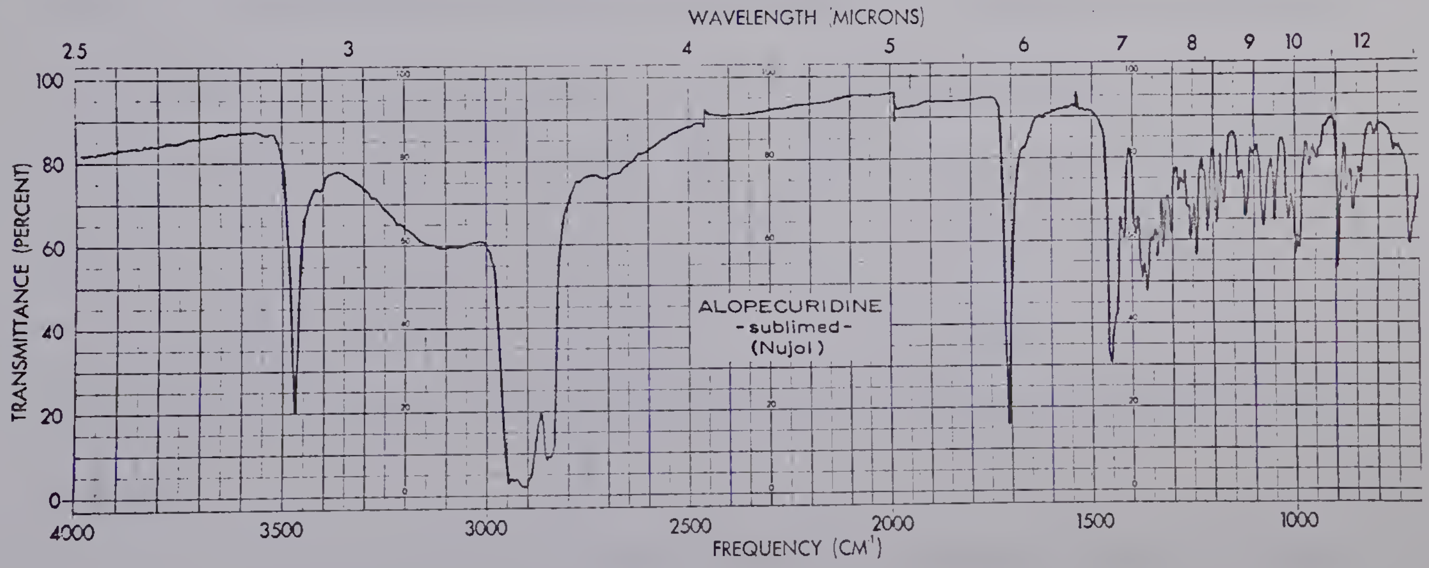


Figure 5

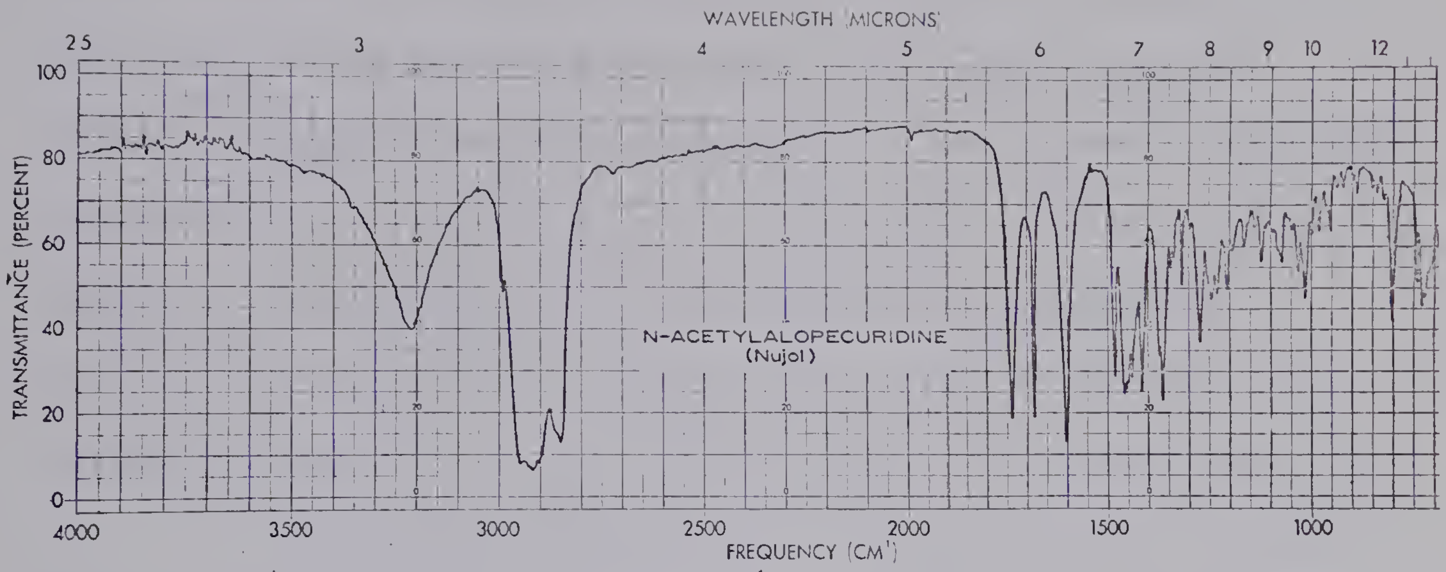
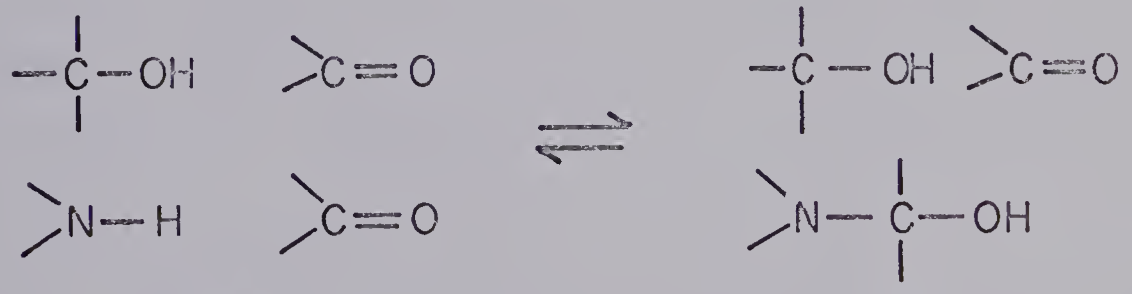


Figure 6

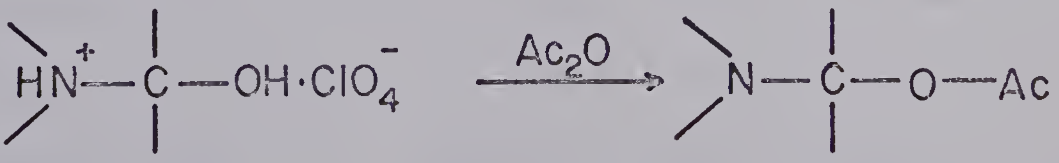
The results could be summarized in terms of the part structures 58 and 59. The chloroform spectrum of the base and the nujol mull spectrum of the crystallized base represents form 58 or an equilibrium mixture of 58 and 59, whereas the sublimed base must be entirely in form 59. The salts are of form 59, but the acetyl derivative is of form 58. The fact that the non-interacting carbonyl group absorbs at different positions (1730 cm^{-1} in the crystallized base, 1710 cm^{-1} in the sublimed base) may be due to varying degrees of intermolecular hydrogen-bonding in the crystal.


58 59

The possibility that one of the carbonyl groups in part structure 58 was an aldehyde was excluded by the fact that the

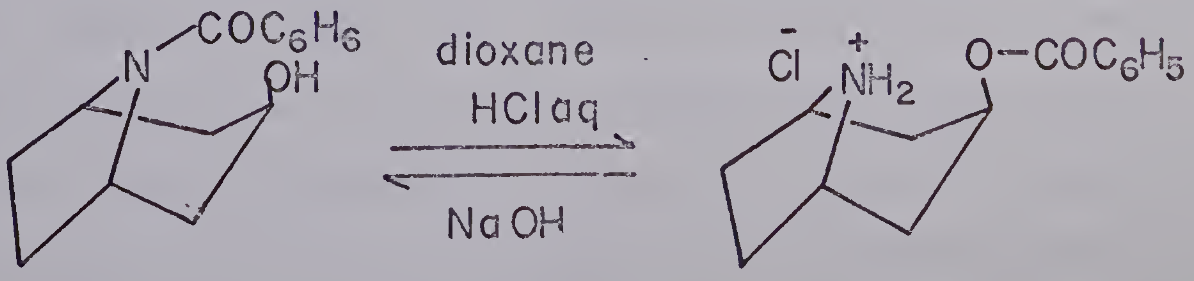
nuclear magnetic resonance spectrum ($CDCl_3$) of N-acetylalopecuridine shows no absorption below τ 6.0. The nuclear magnetic resonance spectrum does show the presence of a secondary C-methyl group at τ 8.92 as well as an acetyl methyl at τ 7.91. It thus appeared that alopecuridine has two ketonic carbonyl groups. One was present in a 8-10 membered ring which contained a trans-annular secondary amino group[55], the other in a cyclopentane ring.

Acetylation of alopecuridine hydroperchlorate (60) with acetic anhydride gave the O-acetyl derivative of the carbinolamine form 59. The mass spectrum of this O-acetyl derivative (61) shows a parent peak at m/e 321 and the infrared spectrum shows hydroxyl absorption, a single carbonyl absorption at 1740 cm^{-1} and an "ester band" at 1240 cm^{-1}. Sublimation of this O-acetyl derivative (61) at 185° effected rearrangement to the N-acetylalopecuridine previously obtained. Fodor[56] has reported the reversible migration of the benzoyl group between amino nitrogen and hydroxyl oxygen in the nortropine system (62) under the conditions shown below:



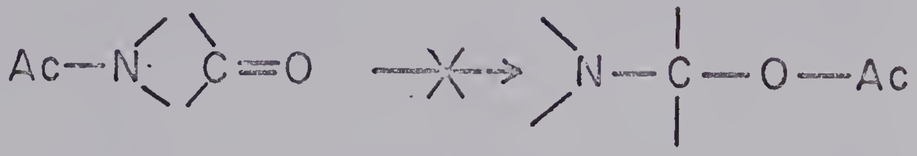
$$\mathrm{H\overset{+}{N}{-}C{-}OH\cdot ClO_4^-} \xrightarrow{Ac_2O} \mathrm{N{-}C{-}O{-}Ac}$$

60 61



62

A similar rearrangement of the acetyl group was attempted by refluxing N-acetylalopecuridine (part structure 63) in acidic solution. After 24 hours none of the O-acetyl derivative (61) could be detected.



63 61

Inspection of the mass spectra of fawcettidine ($M^+ = 245$) (figure 7a) and fawcettimine ($M^+ = 263$) (figure 7b) shows that all the intense peaks of the fawcettidine spectrum at m/e (rel intensity) 245(100), 230(53) and 176(51) are present in the spectrum of fawcettimine. This indicates that fawcettidine is being formed in the mass spectrometer by the dehydration of fawcettimine. In addition to the peaks at m/e (rel intensity) 245(74), 230(70) and 175(88), the mass spectrum of fawcettimine shows peaks at m/e (rel intensity) 233(24), 220(18), 165(62), 150(100), 123(94) and 97(50). All of these latter peaks are present in the mass spectrum of alopecuridine (figure 7c). Besides the peaks at m/e (rel intensity) 233(26), 220(21), 165(71), 150(100), 123(97) and 97(47), the mass spectrum of alopecuridine is devoid of intense peaks. Alopecuridine contains one oxygen more than fawcettimine and the similarity of their infrared and mass spectra suggests that alopecuridine is an hydroxylated fawcettimine. This was confirmed by reduction of alopecuridine with calcium in liquid ammonia to give a mixture of fawcettimine (38) and dihydrofawcettimine (64).

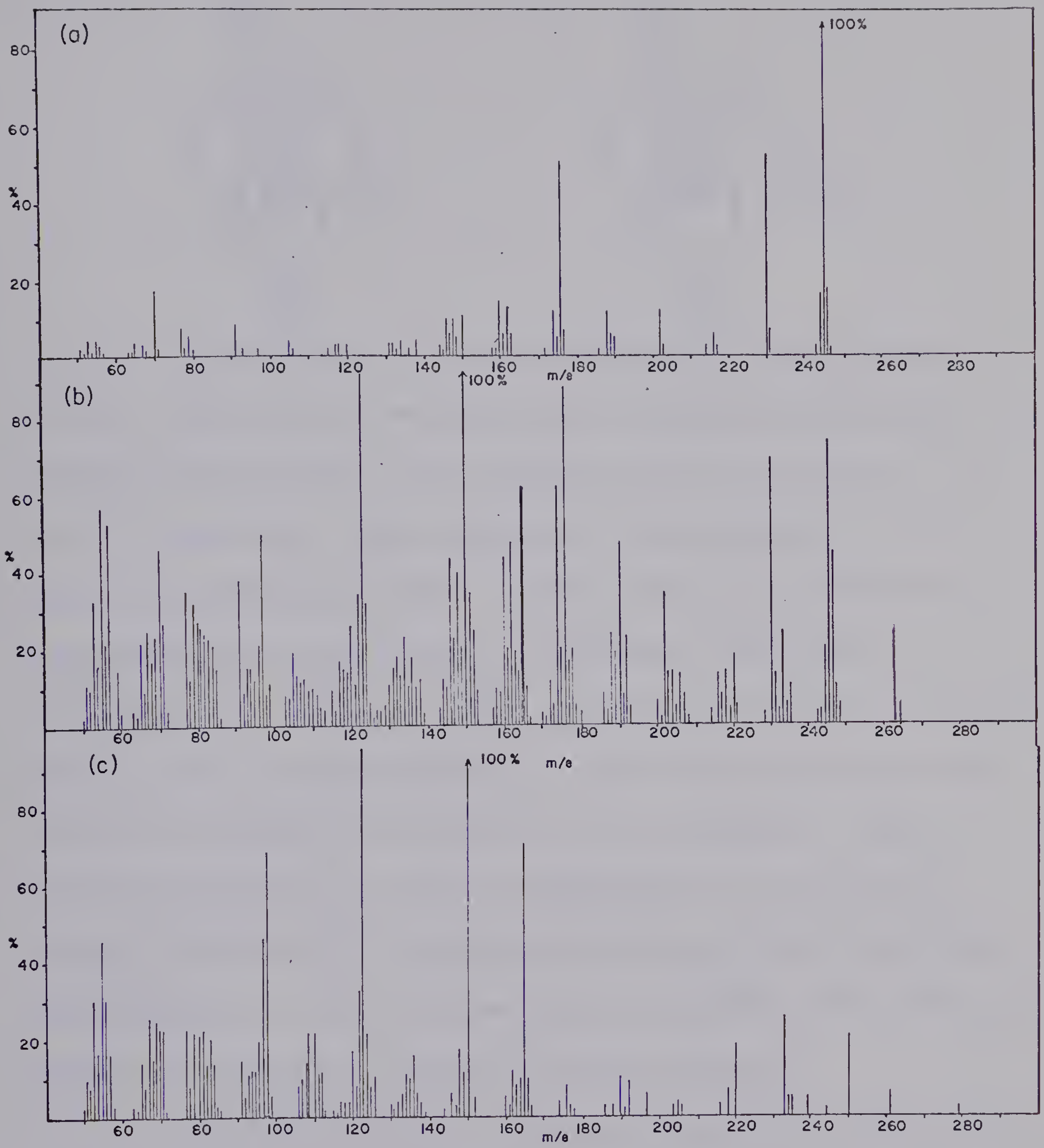
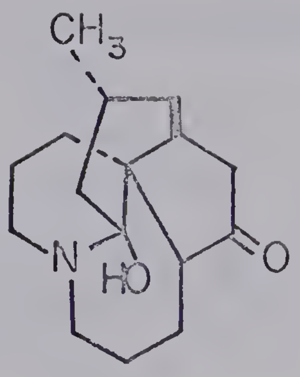


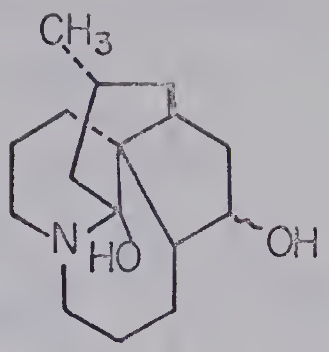
Figure 7 Mass spectrum of (a) fawcettidine

(b) fawcettimine

(c) alopecuridine



38

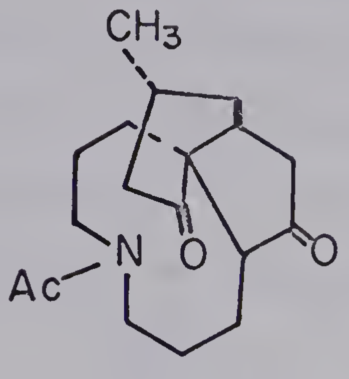


64

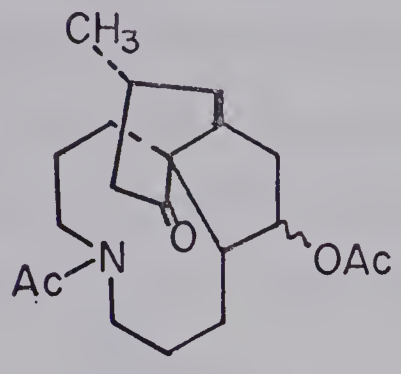
The identity of the fawcettimine was established as follows. The mixture of fawcettimine and dihydrofawcettimine formed in the reduction formed mixed crystals, mp 160-162°, of the two compounds. The melting point and composition on thin-layer chromatography of these crystals remained unchanged on recrystallization from acetone. The mother liquor did not crystallize and showed one main spot on thin-layer chromatography with the same R_f as fawcettimine. The infrared spectrum of this oil was very similar to the spectrum of fawcettimine. When treated with perchloric acid in acetone solution, this oil gave crystals, mp 223-225°, unchanged on admixture with fawcettimine hydroperchlorate. The infrared spectrum of this salt was identical with the spectrum of fawcettimine hydroperchlorate.

The fawcettimine and dihydrofawcettimine of the mixed crystals were separated as their acetyl derivatives. The N-acetylfawcettimine (56), thus obtained, was identical with an authentic sample. The other component melted at 192-194° and was different from O,N-diacetylchanodihydro-8-deoxyserratinine (54), mp 181-183, reported by Inubushi and coworkers[18]. It was assigned

structure 65, the only possible structure for $C_{20}H_{31}NO_4$ (calcd molecular weight 349.2253, found by mass spectrometry 349.2256), that could be derived from alopecuridine and that was consistent with the infrared spectrum, (Nujol) 1735, 1695, and 1640 cm^{-1}.

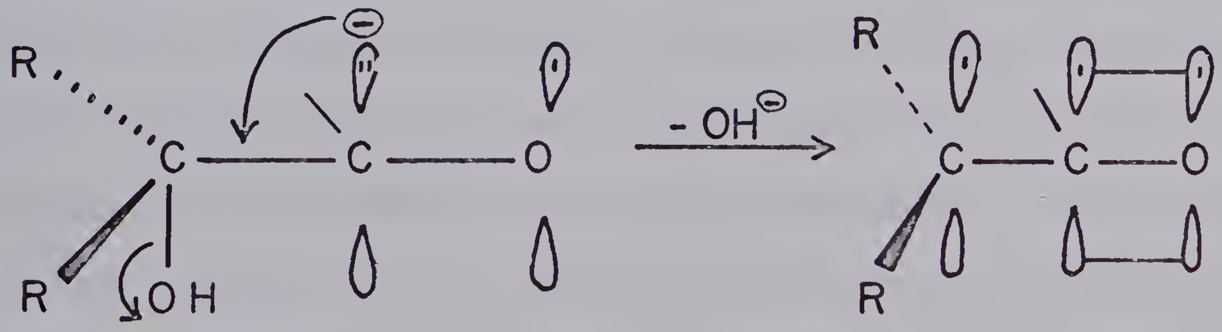


56



65

In order for the elimination of the hydroxyl group adjacent to the carbonyl to have occurred during the calcium-liquid ammonia reduction of alopecuridine it is necessary that the alpha substituent occupies a conformation perpendicular to the plane of the carbonyl group, as shown in structure 66.

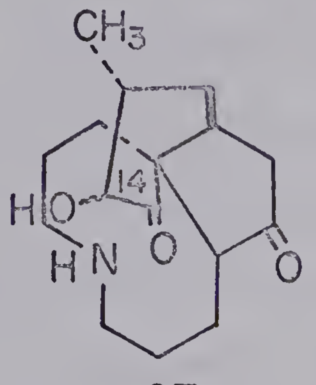


66

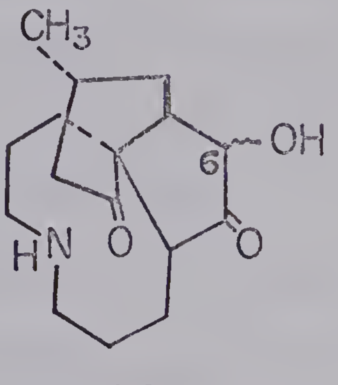
This conformation allows a minimum expenditure of energy for the elimination, since breaking the carbon-substituent bond is accompanied by a continuous overlap of the developing p orbital at the alpha carbon atom with the pi-orbital system of the ion radical[57].

Assuming no skeletal-rearrangement has occured during

the calcium-liquid ammonia reduction of alopecuridine, it has been established that alopecuridine is indeed an hydroxylated fawcettimine and further that the hydroxyl group is adjacent to one of the two carbonyl groups [58]. There are thus three possible structures for alopecuridine, 67, 68 or 69, neglecting for the moment, the carbinolamine form of each structure.

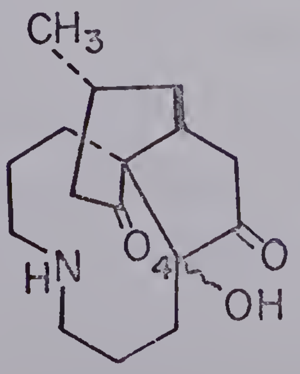


67

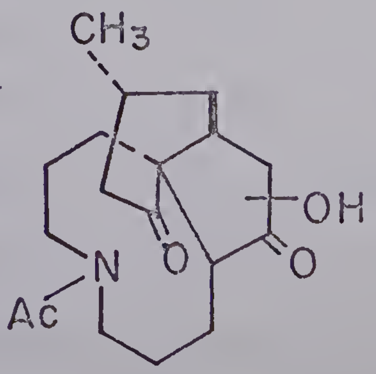


68

The structures 67 and 68 both have a secondary hydroxyl group. The chemical properties of alopecuridine are irreconcilable with such a feature. Attempts to acetylate the hydroxyl group with acetic anhydride in pyridine solution at 95° for 24 hours were unsuccessful. The only product was N-acetylalopecuridine. Attempted Jones' oxidation of N-acetylalopecuridine (70) gave back starting material. No reaction was observed with selenium dioxide in refluxing dioxane.



69



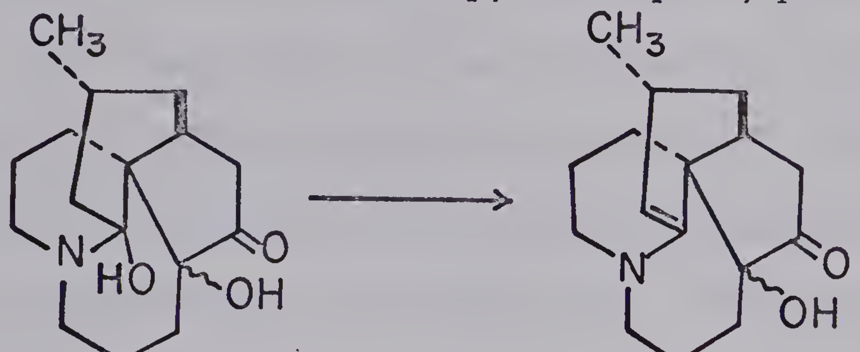
70

This general lack of reactivity of alopecuridine would be more consistent with structure 69 which contains a tertiary hydroxyl group at C-4. However, various attempts made to confirm this structure were inconclusive and the final proof is yet to be made. Experiments which met with limited success will now be discussed.

Examination of the infrared spectra of sublimed alopecuridine, alopecuridine hydrobromide, and alopecuridine hydroperchlorate revealed absorptions in the 1400-1420 cm^{-1} region. These could have been bending frequencies of a methylene group adjacent to a carbonyl. Since each of these compounds exists entirely in the carbinolamine form this would suggest that they have a methylene group at C-6. Confirmation of this would be obtained if these two protons could be exchanged for deuterium. No exchange could be detected (mass spectrometry) either under acidic or basic conditions. An attempt was also made to prepare the benzylidene derivative of alopecuridine. This experiment met with failure.

Attempted dehydration of N-acetylalopecuridine (70) with various reagents gave, usually in poor yield, either starting material or intractable mixtures. Dehydration of N-acetylalopecuridine (70) with thionyl chloride gave a mixture of six products (tlc). This mixture was hydrogenated over platinum and then examined by thin-layer chromatography. One of the components had the same R_f as N-acetylfawcettimine but no separation could be achieved. Two different solvent systems were used to measure the R_f values.

From the results obtained on dehydration of fawcettimine (38) a compound with structure 71 (carbinolamine form of 69) would be expected to dehydrate to give a compound with structure 72. Dehydration of alopecuridine with pyridine-phenylphosphonic dichloride gave two products with similar R_f values. They could not be separated by chromatography over alumina. Partial separation was achieved by glpc at 220°. Only half of the material (20 mg) was recovered from the glpc column. This low yield prevented the recycling of the partially purified compound. The ir, nmr and mass spectral results obtained on this impure compound, did not prove very informative.



71

72

The nuclear magnetic resonance spectrum of N-acetylalopecuridine in dimethyl-$\underline{d}_6$ sulfoxide shows a doublet at τ 4.75 (J = 5 cps) which disappears on the addition of D_2O (the intensity of the spectrum is reduced on addition of D_2O). According to Chapman and King this signal can be assigned to the hydroxyl proton of a secondary hydroxyl group which has been moved downfield by strong hydrogen bonding to the solvent. The reduced rate of proton exchange in dimethyl-$\underline{d}_6$ sulfoxide permits observation of the hydroxyl proton splitting[59, 60].

Until more alopecuridine becomes available, for further investigation of the position and stereochemistry of the hydroxyl group, structure 69 is tentatively proposed for alopecuridine.

The mass spectra of fawcettimine (37) and alopecuridine (69) appear to be complicated by the fact that each alkaloid can exist in two forms (37 ⇋ 38, 69 ⇋ 71) and the carbinolamine form of each (38, 71) dehydrates in the heated inlet. This gives mass spectra that are not amenable to simple rationalization.

The mass spectra of N-acetylfawcettimine (56) and N-acetylalopecuridine (70) are shown in figure 8. A rationalization of the fragmentation of each of these compounds on electron impact is shown in scheme VI.

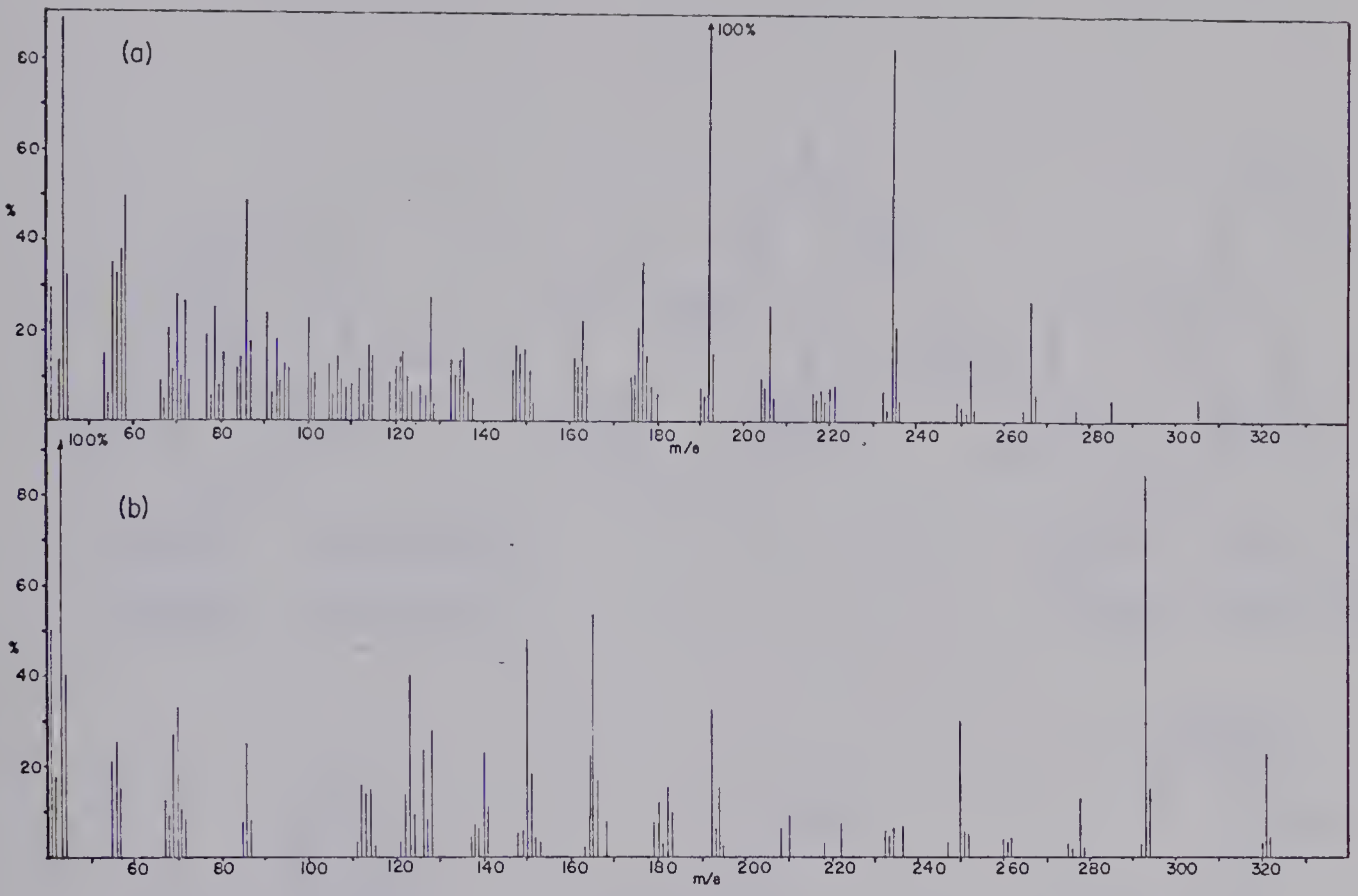
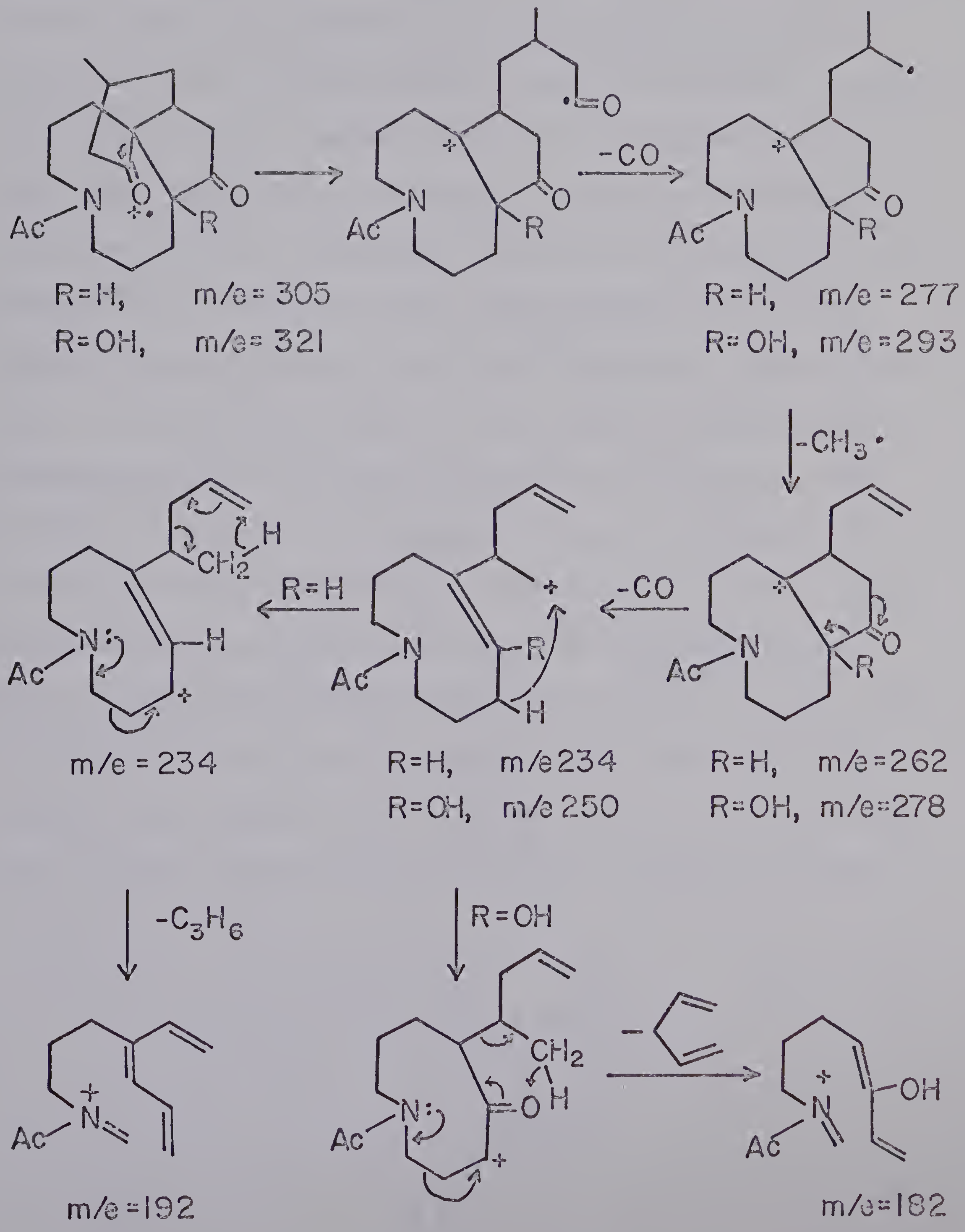


Figure 8 Mass spectrum of (a) N-acetylfawcettimine

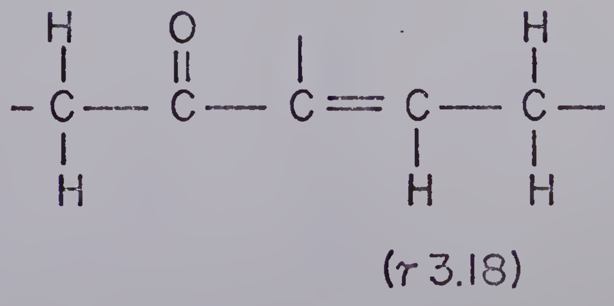
(b) N-acetylalopecuridine

Scheme VI


R=H, m/e= 305
R=OH, m/e= 321
-CO
R=H, m/e=277
R=OH, m/e=293
-CH$_3$•
R=H, m/e=262
R=OH, m/e=278
-CO
R=H, m/e 234
R=OH, m/e 250
R=H
m/e = 234
-C$_3$H$_6$
m/e=192
R=OH
m/e=182

3. The Structure of Alolycopine

An oily component of L. Alopecuroides that shows an absorption in the infrared at 1640 cm^{-1} was first detected in 1965. Hydrobromide, hydrochloride and hydroperchlorate salts were prepared but none of these could be induced to crystallize. Acetylation of the oily base, however, produces a crystalline O-acetylalolycopine, $C_{18}H_{23}NO_3$, that displays characteristic bands in the infrared spectrum at 1740 (acetyl carbonyl), 1715 (carbonyl), 1665 (double bond), 1640 (double bond), 1410 (methylene adjacent to carbonyl), and 1235 (ester C-O-C) cm^{-1} (figure 9). The presence of an α, β-unsaturated ketone in the molecule is indicated by the strong absorption in the ultraviolet spectrum at 237 mμ. At the time this chromophore was thought to account for the bands in the infrared at 1715 (cyclopentenone carbonyl) and 1640 cm^{-1}. The nuclear magnetic resonance spectrum of O-acetylalolycopine (figure 10) shows two olefinic protons at τ 3.18 and τ 4.54. The low-field proton, which shows a medium and a small spin-spin coupling, was assigned to the β-proton of the α, β-unsaturated ketone system as shown in part structures 73 and 74. The other



73

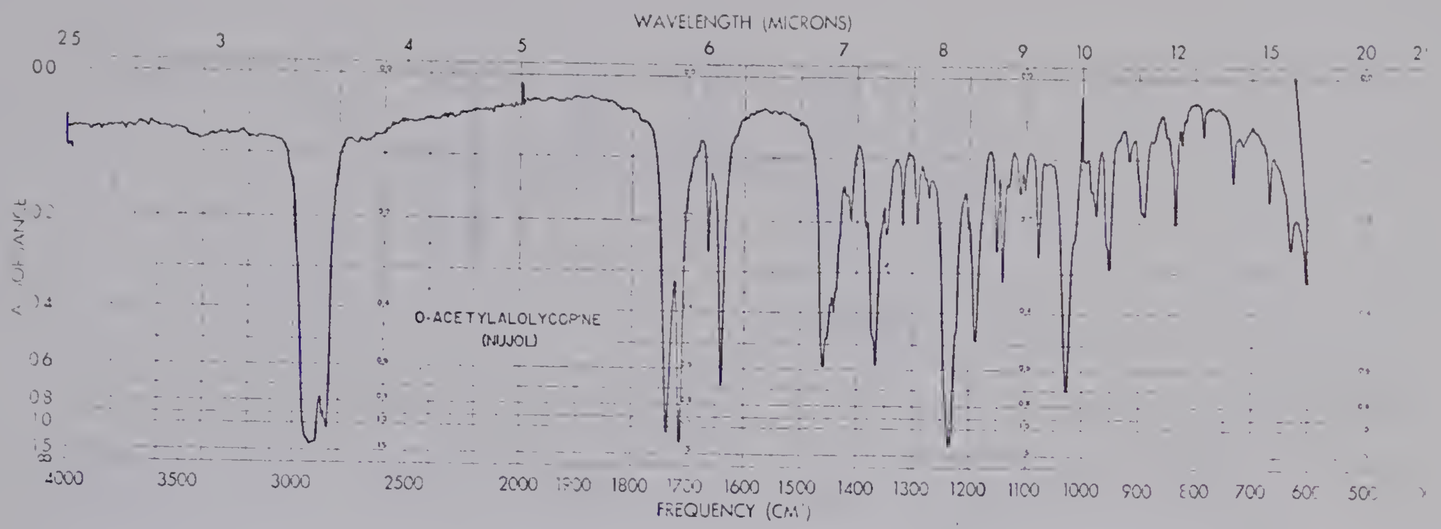


Figure 9

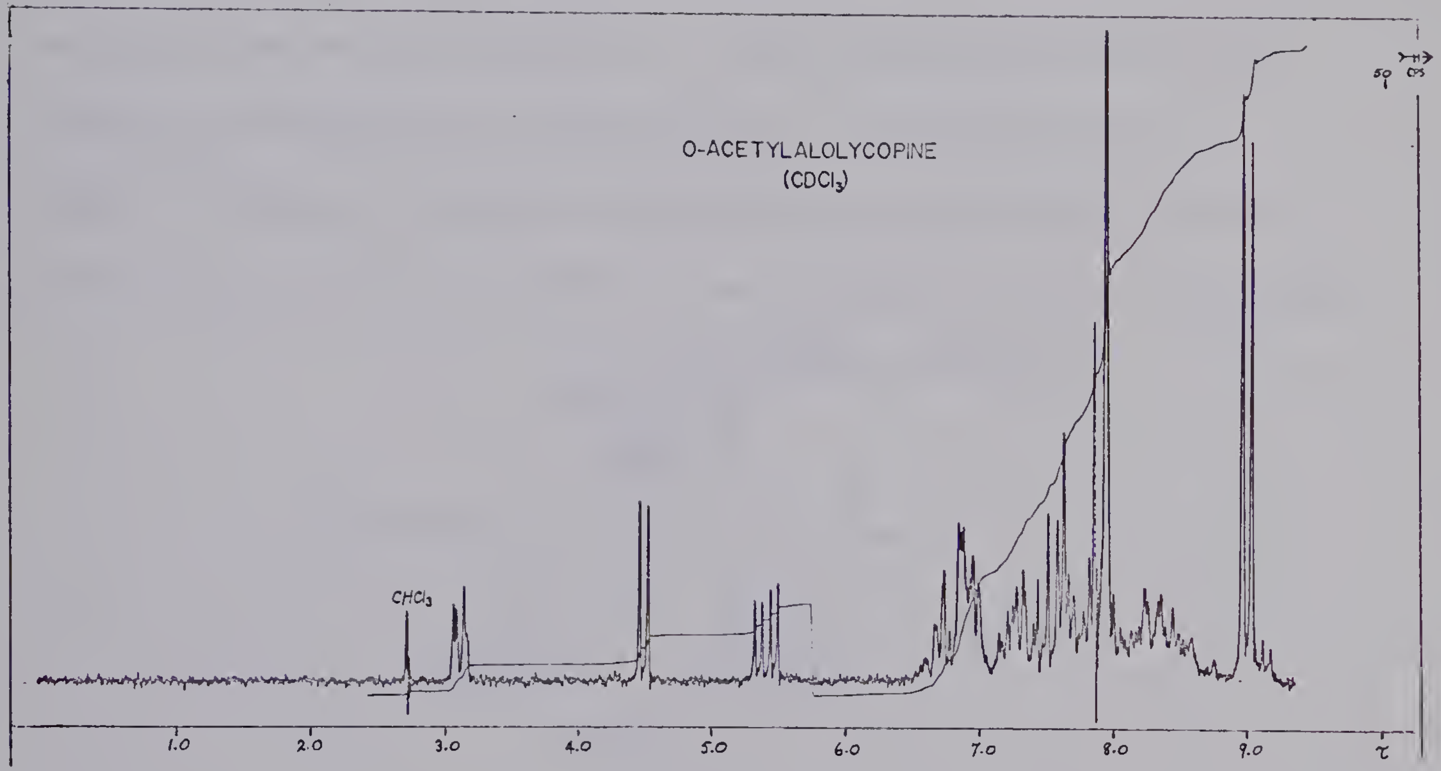


Figure 10

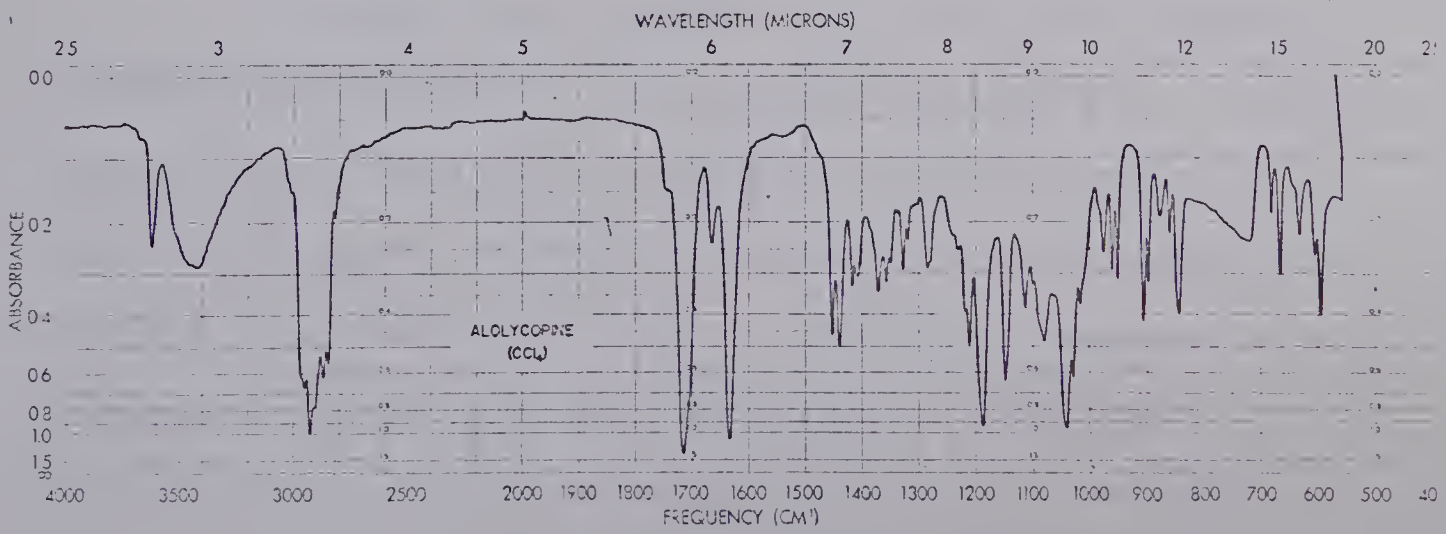
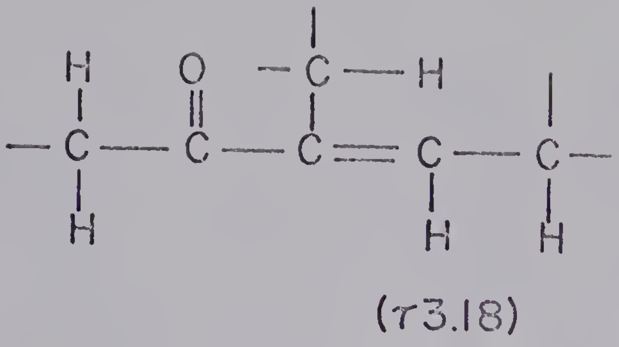
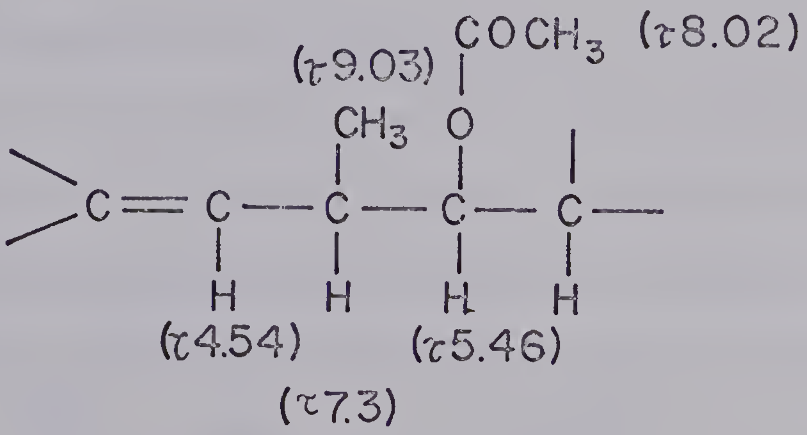


Figure 11



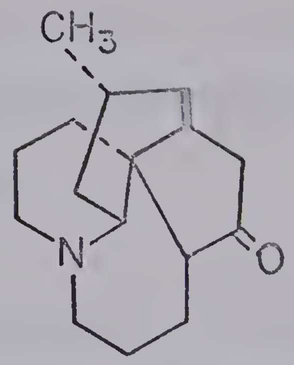
74

olefinic proton, a sharp doublet, was spin decoupled by double irradiation at $\tau 7.3$. Double irradiation at $\tau 7.3$ also spin decoupled the methyl doublet at τ 9.03 and removed one of the spin-spin couplings from the proton at τ 5.46 (quartet, $-\overset{|}{C}\underline{H}-O-COCH_3$). This led to the part structure 75 for O-acetylalolycopine (the chemical shift of each proton is shown in brackets).

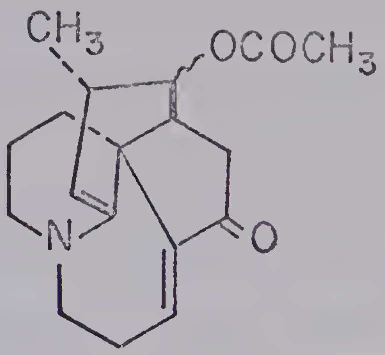


75

Incorporation of the part structures 73 and 75 into the cyclopentanone skeleton 76, common to fawcettidine (40), fawcettimine (37) and alopecuridine (69), produced the tentative structure 77 for O-acetylalolycopine. Structure 74 could not be incorporated into structure 76 without major changes. Structure 77 accounts for all the properties of O-acetylalolycopine and was confirmed by correlation with acetylanhydroaposerratinine (41) a compound



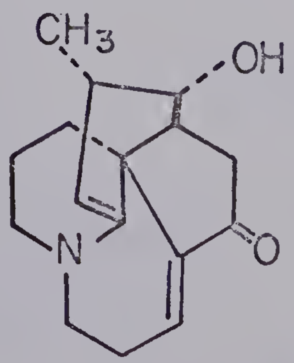
76



77

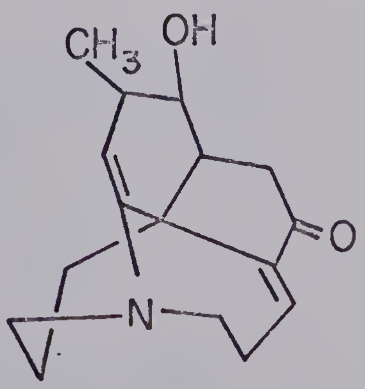
synthesized from serratinine (13) by Inubushi and coworkers[8].

Hydrogenation of O-acetylalolycopine in methanol solution over palladium on powdered charcoal (5%) furnished dihydro-O-acetylalolycopine, purified by chromatography over alumina. Further purification was achieved by molecular distillation. Recrystallization of the distillate from ether gave crystals, mp 144-146°, undepressed on admixture with acetylanhydroaposerratinine (41). The infrared spectra of dihydro-O-acetylalolycopine and acetylanhydroaposerratinine are identical. This unequivocally establishes structure 78 (=78a) for alolycopine.



78

≡



78 a

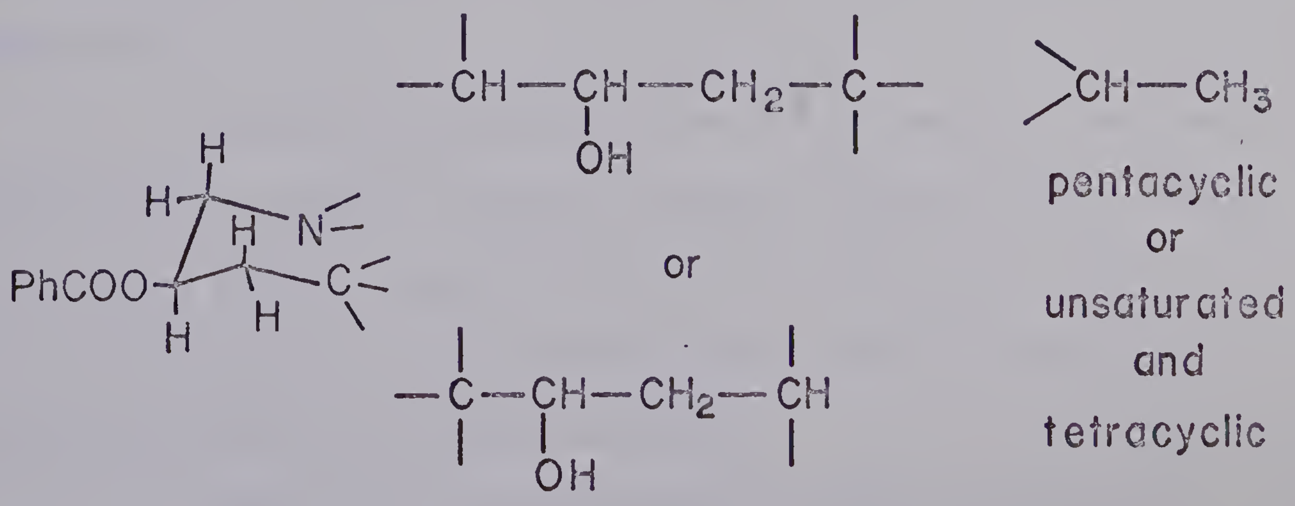
Examination of molecular models of alolycopine shows that the two sides of the double bond of the α, β-unsaturated system are more or less equally hindered to approach of the catalyst.

It therefore seems unwise to assign the stereochemistry at C-4 of acetylanhydroaposerratinine (41) on the basis of the hydrogenation experiment.

Crystalline alolycopine (78) was obtained by the hydrolysis of O-acetylalolycopine (77). Recrystallization from ether furnished a pure sample, mp 53-56°. The infrared spectrum of alolycopine is shown in figure 11. Acetylation of alolycopine (78) gave the O-acetylalolycopine (77) previously described.

4. The Structure of Alopecurine

Alopecurine, a unique *lycopodium* alkaloid, was first isolated in these laboratories by chromatographic separation of crude basic extracts of *L. alopecuroides L**. Preliminary investigations*, which included a detailed study of the spectral characteristics as well as some simple chemical transformations led to the following part structure for alopecurine:



---

* S. Valverde-Lopez, Ph.D. Thesis, U. of A., 1966.

Because of the very small supply of alopecurine the further investigations described here were limited to a few reactions which yielded little new information about the gross structure. It did, however, appear that we were dealing with a new type of alkaloid, since it was impossible to rationalize the results in terms of any of the known skeletons. In view of this we set out to prepare a heavy atom derivative for X-ray diffraction studies. The physical and chemical properties of alopecurine will be discussed in light of the structure of alopecurine as determined by X-ray analysis*.

Four heavy atom derivatives of alopecurine were prepared. The hydrobromide was prepared in methanol and recrystallized from methanol-acetone. Preliminary studies indicated that these crystals were tetragonal, with $a = b = 9.75$ Å and $c = 45.1$ Å (cell volume 4280 Å^3). The very large unit cell together with the unfavorable nature of the tetragonal crystal for X-ray analysis lead to the rejection of this heavy atom derivative of alopecurine.

Likewise the hydrochloride was prepared in methanol with anhydrous hydrogen chloride and recrystallized from acetone. Preliminary studies indicated that it too was tetragonal, with $a = b = 9.54$ Å and $c = 46.0$ Å (cell volume 4180 Å^3) and it was rejected for the same reasons as above.

Alopecurine methobromide was prepared from the

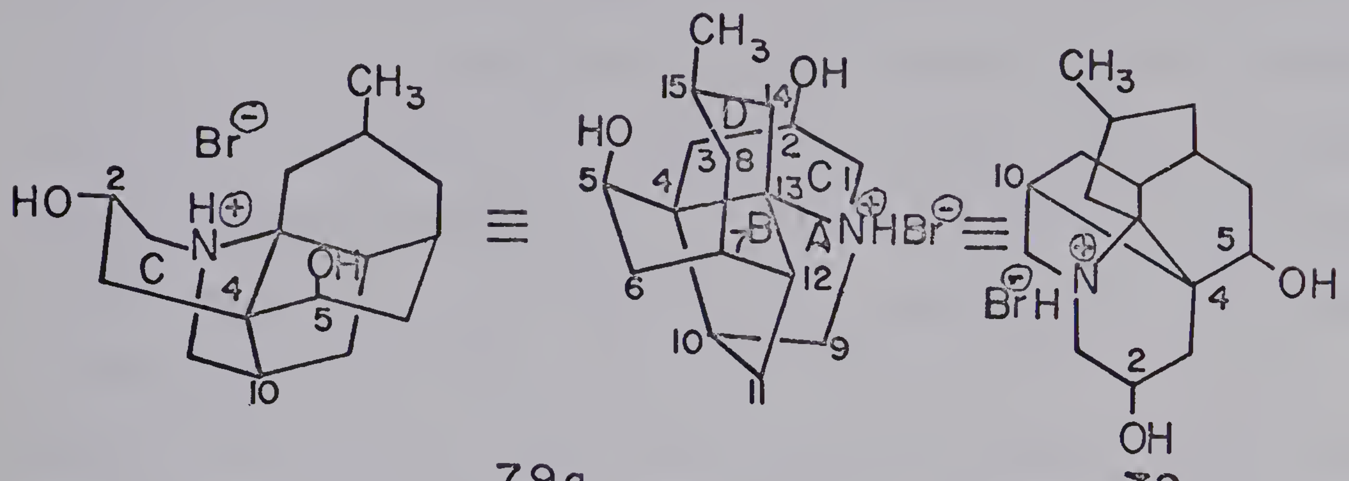
---

* Work done by Dr. N. Masaki, Research Associate, U. of A., 1968.

methiodide which in turn was prepared by refluxing alopecurine with methyl iodide in methanol solution for three hours. An aqueous solution of alopecurine methiodide was washed through a column of Dowex 2-X8 anion exchange resin charged with bromide ions. Evaporation of the water gave crystals of alopecurine methobromide which were repeatedly recrystallized from methanol-acetone until large crystals were obtained. These crystals were monoclinic, space group $P2_1$, with a = 10.96, b = 10.42 and c = 9.49 Å, β = 90° (cell volume 1115 $Å^3$). The calculated density of 1.435 g/cm^3 indicated two molecules per unit cell. Because of the difficulties of applying the heavy atom method to the $P2_1$ space group these crystals were not considered ideal and our attention was directed towards the preparation of heavy atom derivatives of debenzoylalopecurine. Debenzoylalopecurine was isolated from the plant and can easily be prepared from alopecurine by alkaline hydrolysis. Debenzoylalopecurine hydrobromide crystallized from methanol-acetone in the orthorhombic system, space group $P2_12_12_1$, with four molecules of $C_{16}H_{26}BrNO_2$ in a cell of dimensions a = 7.35, b = 28.05 and c = 7.05 Å (cell volume 1450 $Å^3$).

The X-ray reflections were recorded on equiinclination Weissenberg photographs taken along the a and c crystallographic axes with Cu K_α radiation. The structure of debenzoylalopecurine hydrobromide (79a) was solved by the heavy-atom method and refined by the full-matrix least-squares method to an R-factor of 11.9%. Anomalous dispersion calculations allowed the absolute

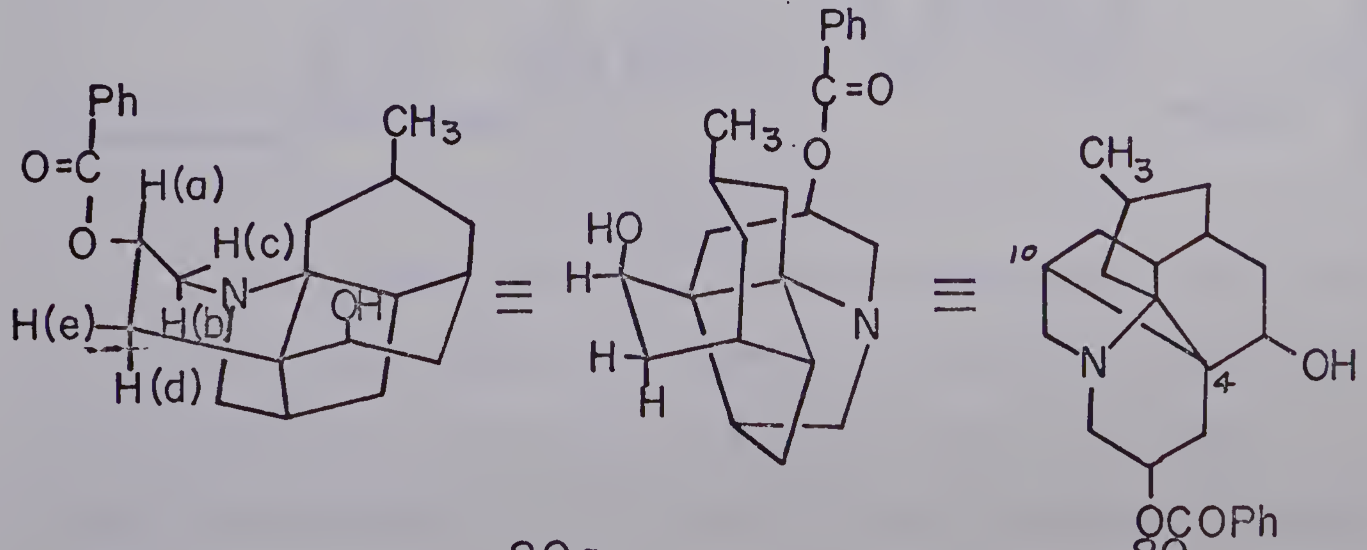
configuration shown in structure 79a to be determined.


Br⊖
HO
2
H⊕
N
C
4
OH
5
10
CH3
≡
CH3
15
14
OH
HO
D
5
3
8
2
4
13
C
1
⊕
B
A
NHBr⊖
7
6
12
10
9
11
≡
CH3
10
5
⊕
N
OH
Br⊖H
4
2
OH

79a 79

The X-ray analysis reveals that debenzoylalopecurine hydrobromide does possess a new carbon skeleton, although as shown in 79 this simply represents an additional bond (between C-4 and C-10) in the lycopodine-type skeleton.

The X-ray analysis also reveals that in the crystal of debenzoylalopecurine hydrobromide (79a) ring C is in the boat conformation. The data on alopecurine itself obtained previous to this investigation are in accord with a structure in which the benzoate group is attached to the "boat-equatorial" hydroxyl group of debenzoylalopecurine, and reveals that ring C is in the boat conformation (80a) in solution as well as in the crystal.


Ph
O=C
O
H(a)
H(c)
N
H(e)
H(b)
H(d)
OH
CH3
≡
Ph
C=O
CH3
O
HO
H
H
H
N
≡
CH3
10
N
OH
4
OCOPh

80a 80

The presence of a benzoyloxy group in alopecurine (80) was indicated by the ultraviolet, infrared, nmr and mass spectra in addition to the fact that benzoic acid was isolated from the alkaline hydrolysis of alopecurine. Thus, the ultraviolet spectrum showed typical benzoate absorption [61] at 230, 272 and 280 m μ; the infrared spectrum of alopecurine (Nujol) exhibited bands at 1705, 1595, 1580, 1270 and 1110 cm^{-1} characteristic of benzoates [62]; the mass spectrum (molecular weight 367) showed strong peaks at m/e 245 (loss of benzoic acid) and at m/e 122, 105 and 77 due to the benzoate portion [63], while the nmr showed absorption in the aromatic region.

The environment and conformation of the benzoyloxy group is clearly defined by the nmr spectrum of alopecurine (80a) (figure 12).

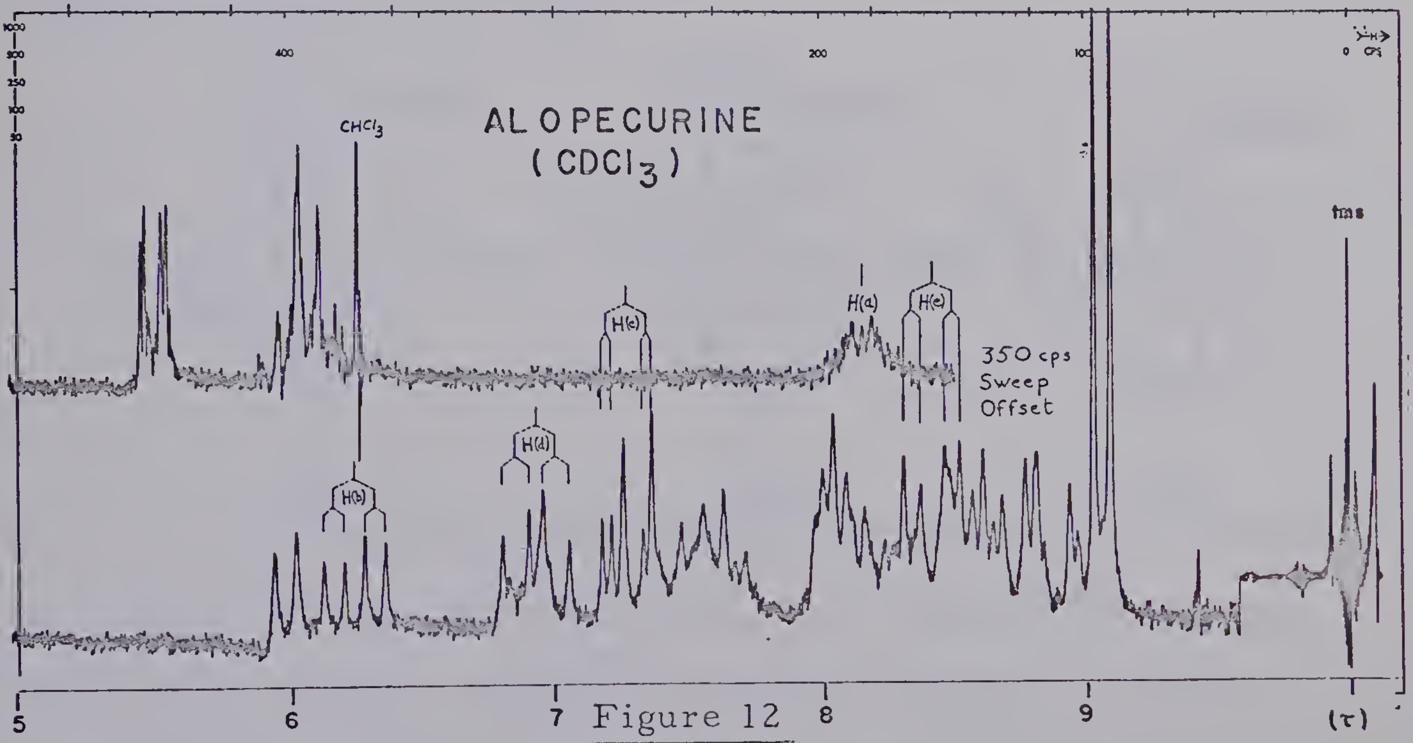
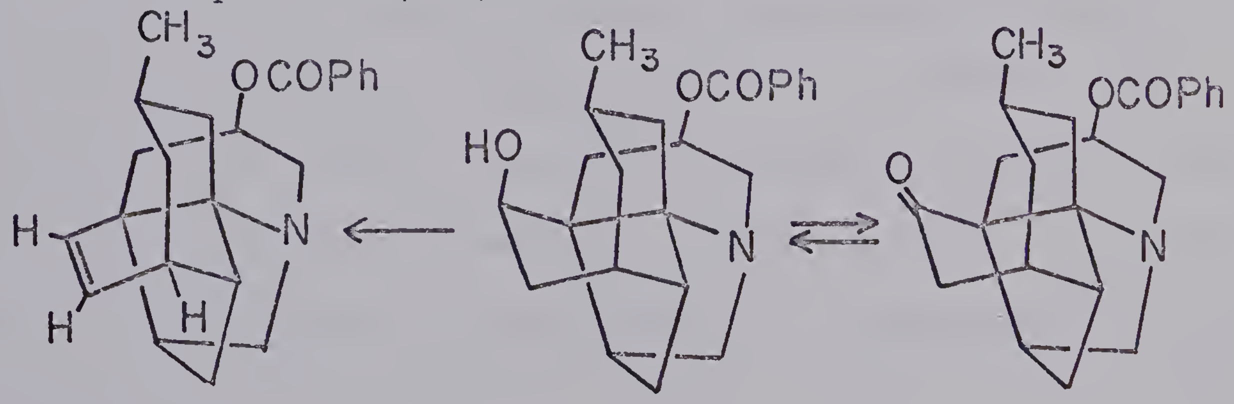


Figure 12

The proton geminal to the benzoyloxy group, H(a) (τ4.15, m, $W\frac{1}{2}$ = 15 cps) is spin-spin coupled to four protons, H(b), H(c), H(d) and H(e) (J_{ab} = 7.5, J_{ac} = 3.5, J_{ad} = 10.0, J_{ae} = 6 cps). Double irradiation at τ4.15 removed all these couplings and each

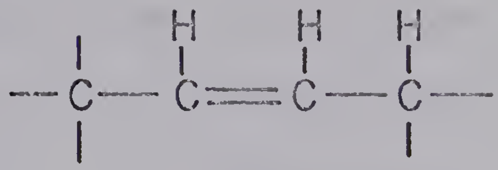
of these four protons now appeared as a doublet (J_{bc} = 15.5, J_{de} = 15 cps). The proton H(a) must exist in the axial configuration to account for the two large couplings [64] (J_{ab} = 7.5 and J_{ad} = 10 cps) present in the quartet signals of H(b) and H(d). Analogous experiments carried out with the O-acetyl derivative of alopecurine confirmed these results. The benzoyloxy group must therefore occupy the equatorial configuration.

Another one proton signal in the nmr spectrum of alopecurine at τ 6.0 (d, J = 8 cps) is assigned to the equatorial proton geminal to the hydroxyl group [65]. This signal shifts downfield (τ 4.8) upon formation of the O-acetyl derivative and disappears from the spectrum of dehydroalopecurine (81 a) obtained by Jones' oxidation of alopecurine (80 a).



82a 80a 81a

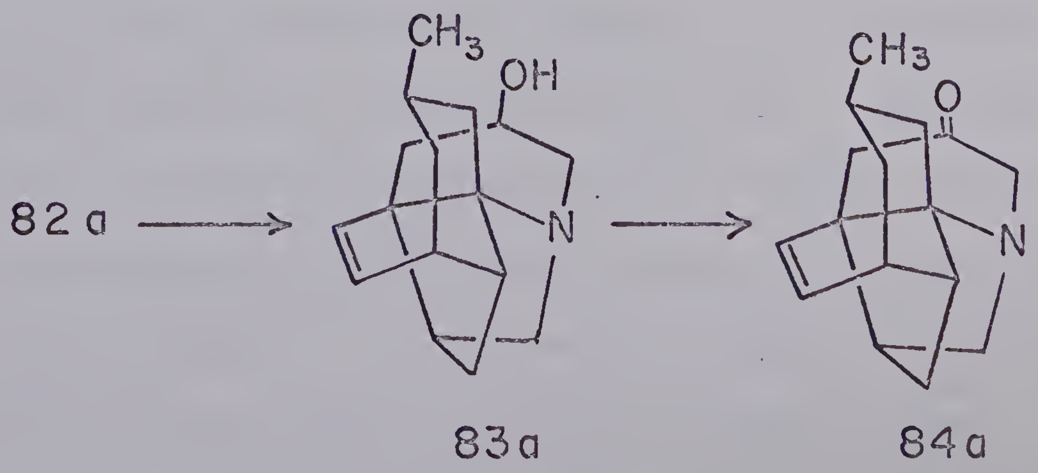
The nmr spectrum of dehydroalopecurine (81 a) retains the signals due to protons H(a), H(b), H(c), H(d) and H(e) present in alopecurine (80 a). Sodium borohydride reduction of dehydroalopecurine (81 a) produced alopecurine in good yield. The nmr spectrum of the anhydro compound 82a shows two olefinic protons at τ 4.5 (1H, d, J = 9 cps) and 4.65 (1H, q, J = 9 and 2 cps) indicating the following part structure:



$$-\overset{|}{\underset{|}{C}}-\overset{H}{\overset{|}{C}}=\overset{H}{\overset{|}{C}}-\overset{H}{\overset{|}{\underset{|}{C}}}-$$

This result is consistent with the environment of the secondary axial hydroxyl group in alopecurine (80a) which could be expected to readily dehydrate [66] in the presence of thionyl chloride and benzene to give anhydroalopecurine (82a).

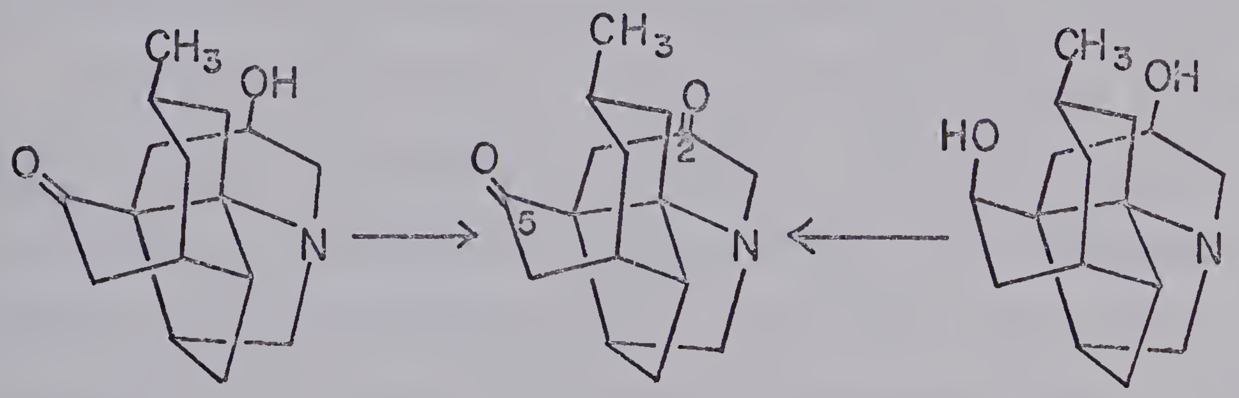
A small amount of anhydroalopecurine (82a) was hydrolyzed with aqueous potassium hydroxide in methanol solution and yielded an homogenous (as determined by tlc) oil that did not crystallize. The infrared spectrum showed the absence of benzoate absorptions and the presence of hydroxyl absorption (3600 cm^{-1}). Jones' oxidation of this anhydrodebenzoylalopecurine (83a) gave another tlc homogenous oil (1 mg) that did not crystallize. The infrared spectrum showed a carbonyl absorption at 1710 cm^{-1} which is in agreement with structure (84a). However, further characterization of compounds 83a and 84a were not carried out due to the lack of material.



Hydrolysis of dehydroalopecurine (81a) with aqueous

potassium hydroxide in dioxane solution gave debenzoyldehydroalopecurine (85a), a crystalline solid with carbonyl absorption (Nujol) at 1690 cm^{-1}. This compound also shows a weak maximum at 225 mμ (ε 1670) which is reduced by half on acidification. That part of the absorption which disappears on protonation of the nitrogen must be due to a σ coupled π interaction[67] between the carbonyl π electrons and the lone pair on nitrogen.

Jones' oxidation of debenzoyldehydroalopecurine (85a) gave a diketone 86a which was the same diketone formed in the oxidation of debenzoylalopecurine (87a).

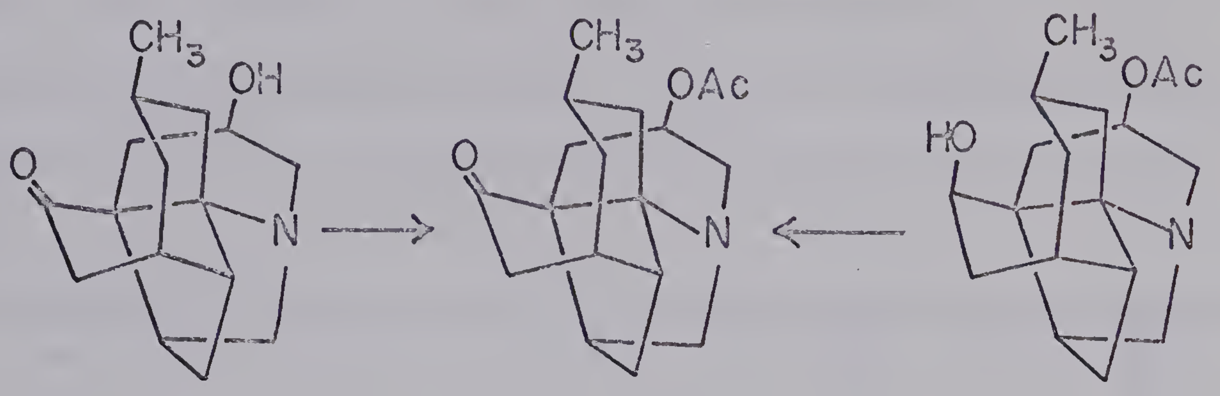


85a 86a 87a

The infrared spectrum of this diketone 86a is in complete agreement with its structure, the carbonyl at C-2 absorbs at 1720 cm^{-1} and the other at C-5 absorbs at 1690 cm^{-1}. The latter value is the same as in debenzoyldehydroalopecurine (85a) and lycopodine (2). Also present in the infrared of this diketone 86a are bands at 1430, 1410 and 1400 cm^{-1} which can be attributed to the three pairs of methylene protons adjacent to the two carbonyl groups.

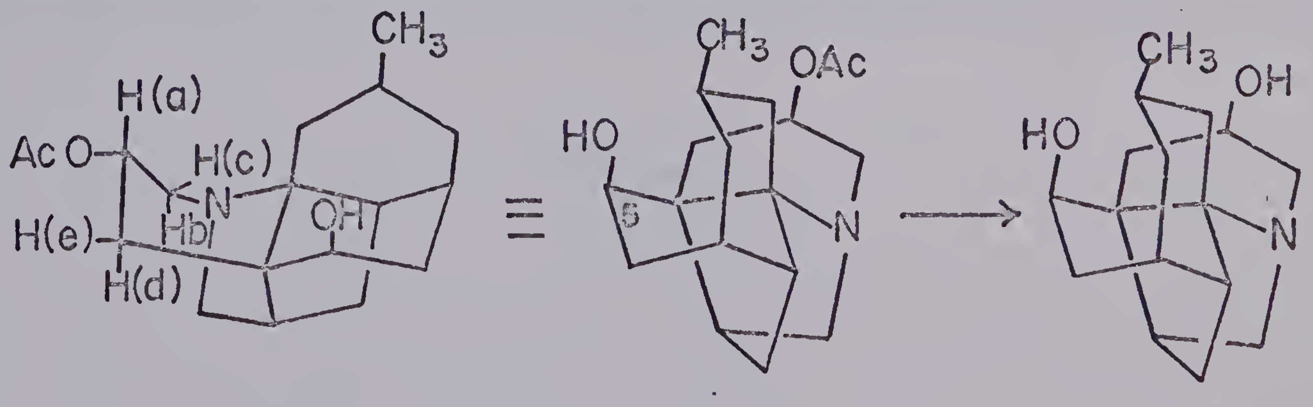
Acetylation of debenzoyldehydroalopecurine (85a) in

pyridine solution with acetic anhydride gave the O-acetyl derivative 88a. This was identical with the product formed on oxidation of acetyldebenzoylalopecurine (89a).



85a 88a 89a

Acetyldebenzoylalopecurine (89a), mp 238-240° is a naturally occuring alkaloid which was isolated from L. alopecuroides L. The above experiment serves to establish the entire structure of 89a except for the stereochemistry of the hydroxyl group at C-5. This hydroxyl was shown to be axial, the same as in alopecurine (80a) by alkaline hydrolysis of 89a. The product obtained was identical in every respect with debenzoylalopecurine (87a).



89a 87a

The nmr spectrum of acetyldebenzoylalopecurine (89a) (figure 13) has been analyzed and found to correspond very closely to that of alopecurine (80a) (figure 12). The chemical shifts of the geminal proton and the four vicinal protons to the acetoxy group are

all at slightly higher field than in alopecurine, while all the coupling constants remain unchanged. The only inexplicable chemical shift is that of H(d) at τ 6.9 in alopecurine (80a) and τ7.0 in acetyldebenzoylalopecurine (89a). There is at present no simple way of explaining the unusually low value of this chemical shift.

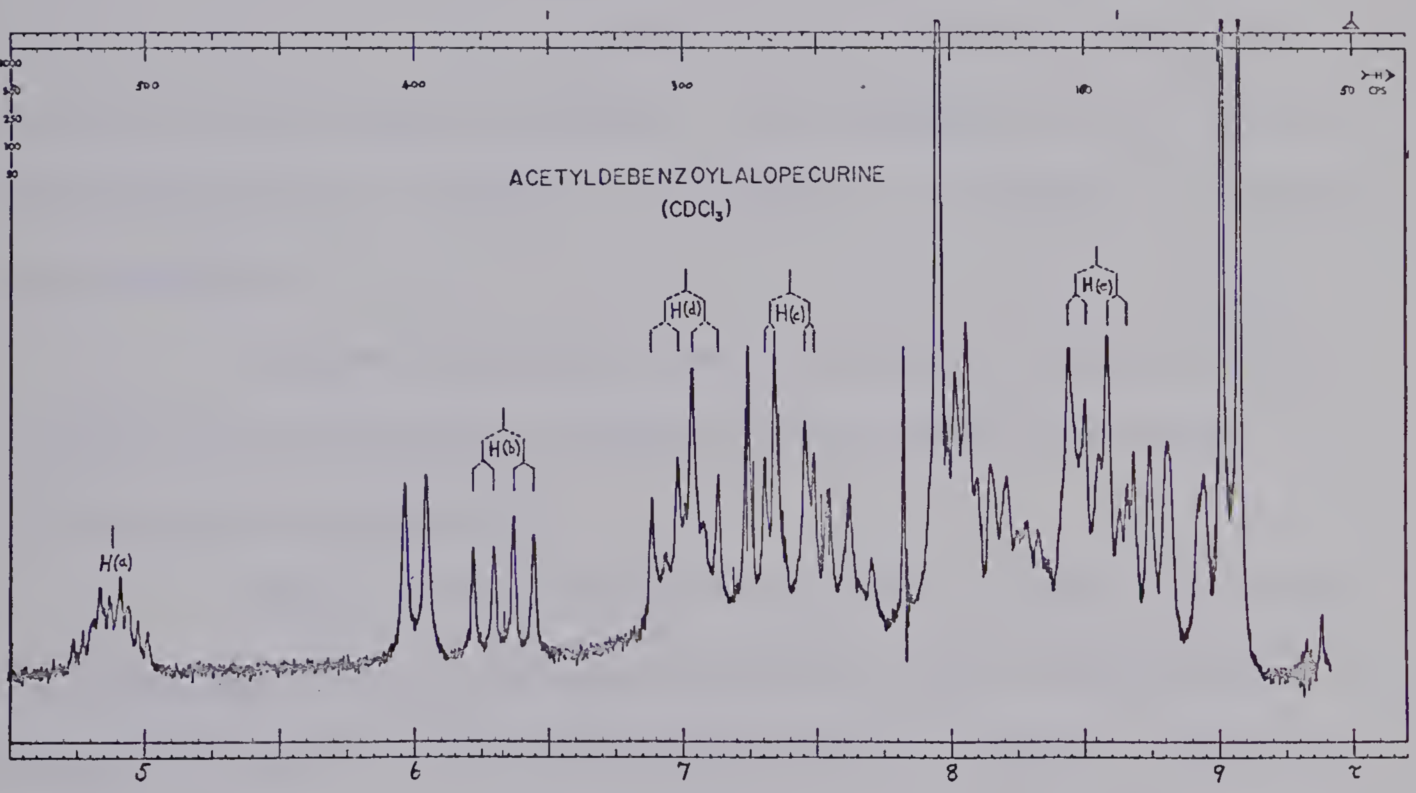


Figure 13

EXPERIMENTAL

GENERAL

Nuclear magnetic resonance (nmr) spectra were measured on a Varian Associates model A-60 or HR-100 spectrometer. Double resonance experiments were performed with the latter instrument. Tetramethylsilane was used as an internal standard unless otherwise stated. The multiplicity of the signals are described as s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet.

Infrared (ir) spectra were recorded on a Perkin-Elmer model 421 dual grating or a Perkin-Elmer model 337 grating infrared spectrophotometer.

Optical rotatory dispersion (ORD), circular dichroism (CD) and most of the ultraviolet (uv) spectra were recorded on a Durrum-Jasco Recording Spectropolarimeter, Model 5. Most of the spectra were recorded in methanol solution in 1 cm, 5 mm and 1 mm cells. The concentrations used for the ORD and CD measurements were about 0.01 M. Some of the ultraviolet spectra were also measured on a Perkin-Elmer model 202 spectrophotometer.

Mass spectra were recorded on an A.E.I. model MS-2H or an A.E.I. model MS-9 mass spectrometer and were recorded as a percentage of the most intense peak (base peak). Unless otherwise stated, the spectra were determined at 70 eV at a temperature between 180-200°.

Melting points were determined on a Leitz Wetzlar hot-stage melting point apparatus and are uncorrected.

Microanalyses were performed by C. Daesslé, Montreal.

Basic alumina refers to Fisher Adsorption Alumina or British Drug Houses (BDH) Alumina of activity III-IV (Brockmann scale). Neutral alumina refers to Woelm neutral alumina of activity I.

Thin-layer chromatograms were carried out on 0.25 mm layers of Alumina G (Research Specialties Co., Richmond, California) and developed in an iodine chamber or with Dragendorff's reagent [68]. The mixed solvent systems used were benzene-ether (1:1); ethyl acetate-methanol (24:1) and chloroform-methanol (49:1) unless otherwise stated.

Gas chromatographic separations were carried out on a Varian Aerograph A-90-P3 using a QF-1 (5 ft) column at 190°. The flow-rate was 60 ml per min and the retention times were between 1 and 10 minutes.

EXPERIMENTAL

PART I

Plant Collection

The material used was collected by Drs. R. K. Godfrey and S. McDaniel in pine flatwoods near Tallahassee, Florida, during September, 1964, and a voucher specimen (No. 5255) is on file in the Florida State University Herbarium.

Separation of the Alkaloids

The crude alkaloids (4.5 g) were placed on a column of basic alumina (150 g) and eluted in 14 fractions as shown in table II.

Fractions 1 and 2 contained non-basic material and were not further investigated.

Fraction 3 showed three spots on tlc, R_f 0.98, 0.90 and 0.5 (benzene-ether). Fraction 3 was combined with similar material from another chromatogram (total, 0.5 g), dissolved in benzene (5 ml) and applied to the top of a dry-packed neutral alumina (250 g) column[43]. The chromatogram was developed with ether (200 ml), the column extruded and the components located by tlc analysis of appropriate aliquots eluted from the alumina with methanol.

The component of R_f 0.9 was obtained as a yellow oil (0.1 g) which slowly turned dark on exposure to air. The mass spectrum was almost identical with that of anhydrolycodoline (25). This material was dissolved in dry ether and converted to the hydrochloride by the addition of anhydrous hydrogen chloride gas.

Table II

Fraction	Eluent	Volume (ml)	Weight of fraction (g)
1	benzene	150	0.02
2	benzene	150	0.06
3	benzene	150	0.16
4	benzene	150	0.15
5	benzene-ether (4:1)	150	0.16
6	benzene-ether (1:1)	150	0.23
7	ether	150	0.10
8	ether	150	0.30
9	ether	150	0.13
10	chloroform	450	0.31
11	chloroform-methanol (99:1)	200	0.25
12	chloroform-methanol (49:1)	200	1.06
13	chloroform-methanol (24:1)	200	0.91
14	chloroform-methanol (9:1)	300	0.29
		Total weight recovered:	4.13 g (92%)

Recrystallization from methanol-acetone afforded white crystals of anhydrolycodoline hydrochloride, mp 274-276°(dec). Identity was established by comparison of the infrared spectrum of the salt with that of an authentic sample and by mixed melting point determination.

The component of R_f 0.5 was isolated in pure form (0.2 g) and shown to be identical with lycopodine (2) in every respect.

The component of R_f 0.98 was obtained as the middle peak in the glpc of fraction 3 (retention time 5 minutes); ir (CCl_4) 1740, 1660, 1415, and 855 cm^{-1}; nmr ($CDCl_3$) τ4.1 (1H, d, J = 5 cps), 6.85 (5H, m), 7.11 (1H, d, J = 8 cps), 7.28 (1H, d, J = 8 cps), and 8.86 (3H, d, J = 7 cps); nmr (CD_3COOD) τ 3.37 (1H, d, J = 5 cps), 6.48 (5H, m), 7.06 (1H, d, J = 7.5 cps, no change after 24 hrs), 7.23 (1H, d, J = 7.5 cps, no change after 24 hrs), and 8.91 (3H, d, J = 6 cps); mass spectrum m/e 160 (14, $C_{11}H_{14}N$), 176 (51, $C_{12}H_{18}N$), 202 (12, $C_{14}H_{20}N$ and $C_{13}H_{16}NO$), 216 (6, $C_{15}H_{22}N$ and $C_{14}H_{18}NO$), 230 (53), and 245 (100, molecular ion). It was treated with an ethereal solution of picric acid and afforded a crystalline picrate (2 mg) mp 220-222°, unchanged on admixture with fawcettidine picrate. The infrared spectra of these two picrates were indistinguishable; ir (Nujol) 1740, 1640, and 1620 cm^{-1}.

Fractions 4, 5, and 6 contained mainly lycopodine. Fraction 7 contained five components (tlc) none of which could be isolated in pure form.

Fraction 8 showed three spots on tlc, R_f 0.98, 0.90 and

0.82 (chloroform-methanol), of which the first was probably lycopodine. The material was dissolved in hot acetone and allowed to cool. Colorless crystals of alopecurine (80) (0.10 g) separated which, after recrystallization from acetone, melted at 244-245° (R_f 0.90). Anal. Calcd for $C_{23}H_{29}NO_3$: C, 76.17; H, 7.95; N, 3.81%. Found: C, 75.52; H, 7.94; N, 3.67%. Mass spectrum m/e 77(50), 105 (100), 122 (19), 151 (19), 178 (26), 204 (25), 219 (38), 230 (21), 245 (59), 246 (30), 262 (55), 352 (22), and 367 (26, molecular ion); ir (Nujol) 3100, 1705, 1595, 1580, 1270, and 1110 cm^{-1}; ir ($CHCl_3$) 3630, 1710, 1605, 1588, 1230, and 1120 cm^{-1}; nmr ($CDCl_3$) τ 4.15 (1H, m, $W_{1/2}$ = 15 cps), 6.0 (1H, d, J = 8 cps), 6.25 (1H, q, J_{bc} = 15.5 and J_{ab} = 7.5 cps), 6.93 (1H, q, J_{de} = 15 and J_{ad} = 10 cps), 7.28 (1H, q, J_{bc} = 15.5 and J_{ac} = 5 cps), 8.43 (1H, q, J_{de} = 15 and J_{ae} = 6 cps), and 9.05 (3H, d, J = 6 cps). Concentration of the acetone mother liquors obtained from fraction 8 yielded crystalline lycodoline (14), mp 179-180°, identical (tlc behaviour, ir spectrum and mixed mp) with an authentic sample.

The evaporation of the solvent from fraction 9 furnished crystals of acetyldebenzoylalopecurine (89a) which after recrystallization from acetone melted at 238-240°. Calcd for $C_{18}H_{27}NO_3$: molecular weight 305.1991. Found: 305.1990 (mass spectrometry). Mass spectrum m/e 178 (40), 204 (46), 219 (51), 245 (100), 246 (60), 262 (50), 290 (58), and 305 (41); ir (Nujol) 1730 and 1250 cm^{-1}; ir ($CHCl_3$) 3610, 1720, and 1240 cm^{-1}; nmr ($CDCl_3$) τ 4.88 (1H, m, $W_{1/2}$ = 18 cps), 6.0 (1H, d, J = 8 cps), 6.33 (1H, q,

J_{ab} = 7.5 and J_{bc} = 15 cps), 7.00 (1H, q, J_{ad} = 10 and J_{de} = 15 cps), 7.40 (1H, q, J_{ac} = 3.5 and J_{bc} = 15 cps), 7.95 (3H, s), 8.54 (1H, q, J_{ae} = 7 and J_{de} = 15 cps), and 9.10 (3H, d, J = 6 cps).

Fraction 10 provided crystals of clavolonine (3), mp 240-241°, identical (tlc behaviour, ir spectrum and mixed mp) with an authentic sample. The mother liquor showed three spots on tlc (chloroform-methanol) corresponding to lycodoline (R_f 0.82), acetyldebenzoylalopecurine (R_f 0.74) and clavolonine (R_f 0.58). These three components were separated by dry-column chromatography as described above except that chloroform-methanol (49:1) was used as developing solvent.

The mother liquors from the clavolonine recrystallization showed a 1640 cm^{-1} band in the infrared spectrum. These were combined with all the mother liquors from fractions 8 and 9 that showed this 1640 cm^{-1} band. A further "dry-column" chromatography was conducted on this material (0.28 g) as described above. From this chromatography a homogeneous oil (tlc) was obtained (R_f 0.6) that could not be induced to crystallize. This oil (0.12 g) was dissolved in pyridine (2 ml), acetic anhydride (2 ml) added, and the mixture was allowed to stand at room temperature for 12 hours. Evaporation of the solvent left an oil which was taken up in ether and filtered through a pad of basic alumina. Slow evaporation of the ether produced crystals (0.10 g) of O-acetylalolycopine (77), mp 180-182°. Calcd for $C_{18}H_{23}NO_3$: molecular weight 301.1678. Found: 301.1678 (mass spectrometry). Mass spectrum m/e

160 (28), 176 (43), 198 (24), 218 (59), 242 (46), 258 (26), 259 (39), 301 (100), and 302 (25); ir (Nujol) 1740, 1715, 1665, 1640, 1410, and 1235 cm^{-1}; uv (MeOH) 237 mμ (ε 6,000); nmr ($CDCl_3$) τ 3.18 (1H, q, J = 6 and 2 cps), 4.54 (1H, d, J = 6 cps), 5.46 (1H, q, J = 12 and 5 cps), 8.02 (3H, s), and 9.03 (3H, d, J = 6 cps).

Hydrolysis of O-acetylalolycopine (0.05 g) was brought about at room temperature in methanol solution (5 ml) with 0.1N aqueous potassium hydroxide (0.5 ml). The chloroform extract of the mixture was dried ($MgSO_4$) and evaporated to give crystalline alolycopine (78) which after recrystallization from ether melted at 53-56°. Calcd for $C_{16}H_{21}NO_2$: molecular weight 259.1572. Found: 259.1571 (mass spectrometry). Mass spectrum m/e 160 (22), 174 (16), 175 (18), 176 (100), 177 (16), 188 (12), 202 (9), 216 (11), 230 (6), 242 (9), 244 (15), and 259 (76); ir (CCl_4) 3620, 3420, 1715, 1665, 1635, and 840 cm^{-1}; uv (MeOH) 237 mμ (ε 4,400).

Fractions 11, 12 and 13 all showed the presence of a component of R_f 0.29 (chloroform-methanol) which crystallized from acetone solutions of these fractions. After recrystallization from acetone the alopecuridine (69) (0.75 g) melted at 171-172°. Further small amounts of alopecuridine were obtained by chromatography of the mother liquors. Anal. Calcd for $C_{16}H_{25}NO_3$: C, 68.79; H, 9.02; N, 5.01%. Found: C, 68.63; H, 8.88; N, 5.31%. Mass spectrum m/e 97 (47, $C_6H_{11}N$), 98 (69, $C_6H_{12}N$), 123 (97, $C_8H_{13}N$), 150 (100, $C_{10}H_{16}N$ and $C_9H_{12}NO$), 165 (71, $C_{10}H_{15}NO$), 218 (7, $C_{14}H_{20}NO$), 220 (21, $C_{14}H_{22}NO$), 233 (26, $C_{15}H_{23}NO$),

261 (7, $C_{16}H_{23}NO_2$), and 279 (3, molecular ion); ir ($CHCl_3$) 3575, 3330, 1740, 1690, and 1595 cm^{-1}; ir (Nujol) 3555, 3430, 3295, 1730, and 1640 cm^{-1}; ir (Nujol-sublimed) 3470, 1710, and 1420 cm^{-1}; nmr (pyridine) τ 9.12 (3H, d, J = 5 cps).

Crystallization of fraction 14 from acetone yielded a small amount (20 mg) of material, R_f 0.08 (chloroform-methanol), mp 230-232° which proved to be identical with the debenzoylalopecurine (87a) described in section 4. The remainder of the fraction could not be separated into pure components.

Oxidation of Dihydrofawcettidine

Dihydrofawcettidine (23 mg) was dissolved in purified acetone (5 ml). To the stirred solution was added 4 drops of Jones' reagent. Methanol (5 drops) was then added and the solution evaporated to near dryness. The residue was dissolved in chloroform (10 ml) and the solution washed twice with dilute aqueous sodium bicarbonate. The dried chloroform solution ($MgSO_4$) was evaporated to yield fawcettidine (40) (18 mg) as an oil.

The experiment was repeated using 38.5 mg of dihydrofawcettidine. The combined yield of fawcettidine weighed 45 mg.

Reduction of Fawcettidine (40)

Fawcettidine (10 mg) was dissolved in methanol (3 ml). Sodium borohydride (5 mg) was added and the solution was allowed to stand overnight at room temperature. Most of the methanol was evaporated and the residue dissolved in water. The aqueous solution was extracted three times with chloroform. The dried chloroform

extracts ($MgSO_4$) were evaporated and the residue (7 mg) taken up in a little acetone (1 ml). On standing the acetone solution deposited crystals, mp 154-156°, of dihydrofawcettidine; ir (Nujol) 3180 (OH), 1660 (C=C), and no carbonyl; nmr ($CDCl_3$) τ 4.47 (1H, d, J = 2 cps), 5.83 (1H, broad multiplet), 7.79 (1H, m), and 8.99 (3H, d, J = 7 cps); mass spectrum m/e 160 (41, $C_{11}H_{14}N$), 176 (59, $C_{12}H_{18}N$), 192 (16, $C_{12}H_{18}NO$), 202 (8, $C_{14}H_{20}N$ and $C_{13}H_{16}NO$), 204 (25, $C_{13}H_{18}NO$ and $C_{14}H_{22}N$), 216 (6, $C_{15}H_{22}N$ and $C_{14}H_{18}NO$), 218 (9, $C_{14}H_{22}NO$ and $C_{15}H_{24}N$), 230 (68), and 247 (100, molecular ion).

Acetylation of Dihydrofawcettidine

Dihydrofawcettidine (10 mg) was dissolved in pyridine (1 ml). Acetic anhydride (1 ml) was added and the solution allowed to stand for 12 hours. The solvent was removed by evaporation and the residue dissolved in chloroform. The chloroform solution was washed with aqueous ammonium hydroxide, dried ($MgSO_4$) and evaporated to give an oil (12 mg). This oil was dissolved in benzene (1 ml) and filtered through a pad of neutral alumina. Evaporation of the benzene gave an homogeneous (tlc) oil which could not be crystallized; ir ($CHCl_3$) 1725 and 1660 cm^{-1}, no hydroxyl absorption.

This O-acetyldihydrofawcettidine was characterized as the hydroperchlorate which was prepared in the usual way and recrystallized from acetone-methanol, mp 219-221° (literature value 220-221°) [54]; ir (Nujol) 1725 and 1660 cm^{-1}.

Conversion of Fawcettimine (38) into Fawcettidine (40)

Fawcettimine* (15 mg) was dissolved in pyridine (2 ml) and an equal volume of phosphorus oxychloride was added. The solution was stirred for 24 hours at 30°. The solution was poured onto ice, made basic (pH > 11) with concentrated ammonium hydroxide and extracted six times with chloroform (20 ml each). The combined dried ($MgSO_4$) chloroform extracts were evaporated to give an oil (4 mg) which was subjected to glpc. The main component (retention time 5 min) was collected (2 mg), the mass spectrum determined and the bulk of it converted into the picrate, mp 220-222°. The mass spectrum thus obtained was very similar to the mass spectrum of fawcettidine. The infrared spectrum of the picrate was identical with the infrared spectrum of fawcettidine picrate. A mixture melting point of 220-222° confirmed the identity.

Alopecuridine Hydroperchlorate

Alopecuridine (10 mg) was dissolved in methanol (2 ml). Perchloric acid (5%) in acetone solution was added until the methanol solution tested acid on litmus paper. The solution was allowed to evaporate at room temperature. After 15 hours crystals had formed. These crystals were washed with acetone and recrystallized from acetone-methanol, mp 224-226°; ir (Nujol) 1750 and 1412 cm^{-1}.

---

* Kindly supplied by Dr. R. H. Burnell.

Alopecuridine Hydrobromide

Hydrobromic acid (fuming) was added to a solution of alopecuridine (10 mg) in methanol (3 ml) until the methanol solution tested acid on litmus paper. The solution was allowed to evaporate at room temperature. After 18 hours crystals had formed. These crystals were washed with acetone and recrystallized from acetone-methanol, mp 230-232°; ir (Nujol) 3200, 3080, 1750, and 1410 cm^{-1}.

Acetylation of Alopecuridine (69)

Acetic anhydride (2 ml) was added to a pyridine (2 ml) solution of alopecuridine (220 mg). The solution was allowed to stand overnight. The solvent was evaporated to leave an oil which was dissolved in chloroform (10 ml). The chloroform solution was washed with aqueous sodium bicarbonate, dilute hydrochloric acid and water. Evaporation of the dried ($MgSO_4$) chloroform solution left a solid which on recrystallization from acetone-methanol gave crystals, mp 223-224°, of N-acetylalopecuridine (70) (144 mg). Calcd for $C_{18}H_{27}NO_4$: molecular weight 321.1940. Found: 321.1939 (mass spectrometry); ir ($CHCl_3$) 3570, 1740, 1690, and 1620 cm^{-1}; ir (Nujol) 3210, 1735, 1680, and 1600 cm^{-1}; nmr ($CDCl_3$) τ 7.91 (s, $C\underline{H}_3CO$) and 8.92 (d, J = 6 cps, $C\underline{H}_3CH$); nmr (DMSO-$\underline{d}_6$) τ 4.72 (1H, d, J = 5 cps), 8.03 (3H, s), and 9.01 (3H, d, J = 5 cps); mass spectrum $\underline{m/e}$ 43 (100), 123 (40), 150 (48), 165 (54), 192 (33), 250 (26), 278 (13), 293 (84), and 321 (22, parent peak).

The experiment was repeated. The solution of

alopecuridine (100 mg) in pyridine (2 ml) and acetic anhydride (2 ml) was heated at 95° for 24 hours. The reaction was worked up as above and gave only N-acetylalopecuridine (56 mg) identical with the compound obtained above.

Acetylation of Alopecuridine Hydroperchlorate (60)

Alopecuridine (22 mg) was dissolved in methanol (5 ml). An acetone solution of perchloric acid (10%) was added until the methanol solution gave an acid test with litmus paper. The solvent was removed by evaporation and the residual solid dissolved in acetic anhydride. This solution was allowed to stand for 5 hours. The solution was evaporated to dryness and the residue dissolved in water. The aqueous solution was extracted three times with chloroform. Evaporation of the dried ($MgSO_4$) chloroform extract gave a solid (5.3 mg) which was recrystallized from acetone, mp 223° (dec); ir (Nujol) 1680 cm^{-1}; uv (EtOH) 220 mμ (ε 12,000) and 240 mμ (sh) (ε 10,000). Chromatography of the mother liquors from this crystallization over basic alumina gave another compound, recrystallized from acetone, mp 183-184°; ir (Nujol) 1760 cm^{-1}; uv (MeOH) 240 mμ (ε 11,000) and 300 mμ (ε 10,000). These neutral compounds formed by the acid catalyzed self condensation of acetic anhydride, have previously been reported by Praill and Whitear[69].

The aqueous extract was made basic with dilute ammonium hydroxide (pH > 11) and extracted with chloroform. The chloroform extract was dried ($MgSO_4$) and evaporated to give an oil (16 mg).

This oil was purified by molecular distillation (165°, 0.5 mm Hg); ir ($CHCl_3$) 3560, 1740, and 1240 cm^{-1}; mass spectrum m/e 261 (12), 262 (100), and 321 (2, parent peak).

Molecular distillation of this O-acetylalopecuridine (61) at a higher temperature (185°, 0.5 mm Hg) produced a product that was identical with N-acetylalopecuridine (70). Comparison was made by mp, mmp and ir spectrum.

Attempted Rearrangement of N-Acetylalopecuridine (70)

N-Acetylalopecuridine (9 mg) was dissolved in acetone (3 ml). Hydrochloric acid (2N) (0.5 ml) was added and the solution refluxed for 24 hours. The solvent was removed by evaporation and the residue dissolved in chloroform. The chloroform solution was washed with water, dried ($MgSO_4$), and evaporated to give N-acetylalopecuridine (7 mg) identical with an authentic sample. The aqueous layer was basified with concentrated ammonium hydroxide and extracted with chloroform. The dried ($MgSO_4$) chloroform extract was evaporated and left no residue.

Reduction of Alopecuridine (69) with Calcium in Liquid Ammonia

Small pieces of calcium metal were added to a stirred solution of alopecuridine (78 mg) in liquid ammonia (25 ml). The addition of calcium was stopped when the solution remained blue. The stirring of the solution was continued for a further 5 minutes. Ammonium chloride (0.5 g) was added to the solution and the ammonia allowed to evaporate. When most of the ammonia had evaporated water was added and the aqueous solution extracted with

chloroform. Evaporation of the dried ($MgSO_4$) chloroform extract furnished a solid that was recrystallized from acetone. The crystals, mp 160-162°, and mother liquor of this crystallization were treated separately.

The mother liquor, examined on tlc, showed one main component with the same R_f as fawcettimine (37) (R_f 0.55, ethyl acetate-methanol). The solvent was removed from the mother liquor by evaporation. The infrared spectrum of the resulting oil was very similar to the spectrum of fawcettimine; ir (CCl_4) 3585, 1732, and 1410 cm^{-1}. The bulk of the oil(28 mg) was converted into the crystalline hydroperchlorate by neutralization with perchloric acid in acetone solution. Recrystallization from acetone-methanol gave crystals (19 mg), mp 224-226°; ir (Nujol) 3400, 1700, and 1410 cm^{-1}. A mixture melting point with fawcettimine hydroperchlorate was 223-226°. The infrared spectra of this hydroperchlorate and fawcettimine hydroperchlorate were identical.

The crystals (32 mg), mp 160-162°, were repeatedly recrystallized from acetone. The melting point remained unchanged. Examination of these crystals by tlc showed two components. The composition of the mixture by tlc remained unchanged after each recrystallization. Most of the crystals (30 mg) were dissolved in pyridine (2 ml). Acetic anhydride (2 ml) was added and the solution allowed to stand overnight. The solvent was removed by evaporation and the residue dissolved in chloroform (10 ml). The chloroform solution was washed with aqueous sodium bicarbonate,

dilute hydrochloric acid and water, dried ($MgSO_4$) and evaporated. The residual solid (25 mg) was composed of two compounds (tlc). They were separated by chromatography over alumina (1.0 g). Elution with ether furnished a compound (65), mp 192-194°, $C_{20}H_{31}NO_4$ (calcd molecular weight 349.2253, found by mass spectrometry 349.2256); ir (Nujol) 1735, 1695, and 1640 cm^{-1}; mass spectrum m/e 176 (22), 188 (25), 218 (25), 230 (22), 246 (45, $C_{16}H_{24}NO$), 248 (27, $C_{16}H_{26}NO$), 289 (100, $C_{18}H_{27}NO_2$), 290 (45, $C_{18}H_{28}NO_2$), 306 (22), and 349 (4, molecular ion, $C_{20}H_{31}NO_4$). Elution with chloroform-ether (1:1) furnished a compound (56), mp 143-145°; ir (Nujol) 1740, 1695, and 1620 cm^{-1}; mass spectrum m/e 128 (27, $C_7H_{14}NO$), 177 (36, $C_{12}H_{17}O$), 192 (100, $C_{12}H_{18}NO$), 205 (26), 234 (82, $C_{15}H_{24}NO$), 262 (14, $C_{16}H_{24}NO_2$), 277 (38, $C_{17}H_{27}NO_2$), and 305 (3, parent peak). A mixture melting point with N-acetylfawcettimine, 143-145°, confirmed the identity of this compound.

Attempted Oxidation of N-Acetylalopecuridine (70) with Jones' Reagent

N-Acetylalopecuridine (71 mg) was dissolved in acetone (10 ml). Jones' reagent (10 drops) was added to the stirred solution. After a few minutes methanol (10 drops) was added and the solution evaporated to near dryness. The residue was dissolved in water (5 ml) and extracted with chloroform. The chloroform solution was dried ($MgSO_4$) and evaporated to give a solid (59 mg). Recrystallization from acetone-methanol gave crystals, mp 222-224°, mixture

melting point with N-acetylalopecuridine 222-224°. The infrared spectra of this product and N-acetylalopecuridine were identical.

Attempted Oxidation of N-Acetylalopecuridine (70) with Selenium Dioxide

N-Acetylalopecuridine (32 mg) was dissolved in a mixture of dioxane and ethanol (2:1) (20 ml). Freshly sublimed selenium dioxide (20 mg) was dissolved in water (1 ml) and added to the solution. The solution was refluxed for 12 hours and then filtered. The volume of the filtrate was reduced to 5 ml by evaporation, basified with concentrated ammonium hydroxide and extracted with chloroform. Evaporation of the dried ($MgSO_4$) chloroform solution gave a solid (30 mg) that was recrystallized from acetone-methanol, mp 222-224°. The infrared spectra of this product and N-acetylalopecuridine were identical.

Attempted Deuterium Exchange

Alopecuridine (12 mg) was dissolved in methanol-$\underline{d}_1$ (1 ml). Sodium (20 mg) was added and the solution refluxed for 12 hours under a dry nitrogen atmosphere. The solvent was evaporated and the residue dissolved in deuterium oxide (1 ml). The deuterium oxide solution was extracted with purified methylene chloride. The methylene chloride solution was dried ($MgSO_4$) and evaporated to give a solid. The mass spectrum of this solid was almost identical with the mass spectrum of alopecuridine. Mass spectrum $\underline{m/e}$ 123 (95), 150 (90), 165 (100), 233 (40), 261 (8), 262 (9), and 279 (2).

Alopecuridine hydrobromide (15 mg) was dissolved in deuterium oxide (3 ml), one drop deuterium bromide solution added, and the solution was refluxed for 24 hours. The deuterium oxide was evaporated and the infrared spectrum of the crystalline solid determined. The infrared spectrum showed that only the hydroxyl proton had exchanged; ir (Nujol) 3100, 3080, 2400, 2300, 1750, and 1410 cm^{-1}. The experiment was repeated and the reflux time increased to 49 hours. Sodium carbonate (anhydrous) was added to the deuterium oxide solution until the solution tested basic on litmus paper. The basic solution was extracted with purified methylene chloride. The methylene chloride solution was dried ($MgSO_4$) and evaporated to give a solid. The mass spectrum of this solid was almost identical with the mass spectrum of alopecuridine.

Attempted Benzylidene Formation

Alopecuridine (20 mg) was dissolved in 5 ml methanol. Sodium (25 mg) and benzaldehyde (0.25 ml) were added. The mixture was refluxed for 3 hours, cooled, concentrated under vacuum and diluted with water (5 ml).

The aqueous solution was acidified with dilute hydrochloric acid (2N) and extracted with ether. The aqueous layer was basified with concentrated ammonium hydroxide and extracted with chloroform. The dried ($MgSO_4$) chloroform extract was evaporated to give a solid (13 mg) (homogeneous by tlc) which was identical with alopecuridine.

Dehydration of N-Acetylalopecuridine (70)

Thionyl chloride (1 ml) was added to a solution of N-acetylalopecuridine (50 mg) in methylene chloride (3 ml). The solution was refluxed for one hour and then cooled. Water (1 ml) was added slowly and the solvent removed by evaporation. The residue was dissolved in chloroform and washed with water. The chlorform solution was dried ($MgSO_4$) and evaporated to give an oil, which showed six components by tlc, one of which was the starting material. Chromatography of the oil over alumina (1.5 g) eluting with benzene separated the less polar products from the more polar starting material. The less polar products (10 mg) showed five components by tlc in about equal proportion.

Hydrogenation of this mixture of products with Adams' catalyst at atmospheric pressure gave an oil that was examined by tlc. It showed many spots, one of which was indistinguishable from N-acetylfawcettimine (56). A large tlc plate (8" x 8") was used for the comparison and developed in one direction with chloroform-methanol (19:1) and across the plate with ethyl acetate-methanol (12:1).

The experiment was repeated with another sample of alopecuridine (60 mg) and the reflux time was extended to three hours. The nonpolar products of the reaction showed five components by tlc and weighed 12 mg. Chromatography over alumina, preparative tlc and glpc were used to separate the components of this mixture. In no case was a pure component isolated from the

mixture.

Dehydration of Alopecuridine (69)

Phenylphosphonic dichloride (5 ml) was added to a pyridine solution (5 ml) of alopecuridine (80 mg). The solution was stirred for 19 hours at room temperature and then poured onto ice. The aqueous solution was basified (pH > 11) with concentrated ammonium hydroxide and extracted with chloroform. The dried ($MgSO_4$) chloroform solution was evaporated to give an oil (40 mg). Examination of this oil by tlc showed a major product, a minor product, and some starting material. The major and minor products were separated from the alopecuridine by chromatography over alumina (1.0 g, elution with benzene). The major and minor products had very similar R_f values and could not be separated from each other by chromatography over alumina. The major product was separated by glpc at 220° (retention time 3 min). It was an oil (20 mg) that showed one spot by tlc, but its purity was open to question because of the infrared spectrum, ir ($CHCl_3$) 3500, 1740, 1700, and 1660 cm^{-1}. The major product was contaminated by the minor product which was obtained from the glpc (retention time 6 min) as a crystalline solid (3 mg), mp 129-130°; ir (Nujol) 1730, and 1690 cm^{-1}; uv (MeOH) 272 mμ (ε 4,800); mass spectrum m/e 123 (88), 150 (77), 165 (100), 233 (50), and 261 (7, parent peak).

The nmr of the impure major product was determined, nmr ($CDCl_3$) τ 4.35 (1H, broad singlet, $W_{1/2}$ = 3.5 cps) and 8.93 (3H, d, J = 6 cps). Double irradiation at τ 7.74 gave nmr ($CDCl_3$)

τ 4.35 (1H, sharp singlet) and 8.93 (singlet). The oil was recovered from the deuterochloroform solution and again subjected to glpc under the same conditions. The recovered oil (8 mg) had the same R_f as before; ir (CCl_4) 3500, 1740, and 1650 cm^{-1}; mass spectrum m/e 150 (70), 160 (100), 165 (70), 233 (50), 243 (60), and 261 (65, parent peak). The infrared spectrum of this sample remained unchanged on recycling the sample through the glpc and the sample was considered to be pure.

Hydrogenation of O-Acetylalolycopine (77)

O-Acetylalolycopine (25 mg) in methanol solution (30 ml) was stirred in the presence of palladium on powdered charcoal (5%) (25 mg) under hydrogen at atmospheric pressure for two hours. The catalyst was removed by filtration and the solvent evaporated to leave a solid that showed one major and three minor components by tlc. The solid was adsorbed on basic alumina (1 g) and eluted with ether in five 10-ml fractions. Fractions 2, 3, and 4 were combined (12 mg) and recrystallized from ether, mp 142-145°. Further purification by molecular distillation (75°, 0.5 mm Hg) gave a solid that was recrystallized from ether, mp 144-146°, ir (Nujol) 1735 (cyclopentanone and acetyl carbonyl), 1660 (C=C), 1415 ($-CH_2CO-$), and 1230 cm^{-1} (CH_3CO-O-, "ester band"); mass spectrum m/e 160 (60), 176 (100), 188 (33), 244 (83), 260 (25), 288 (16), and 303 (66, parent peak). The mixture melting point with acetylanhydroaposerratinine (41) was 144-147°. The infrared spectra of dihydro-O-acetylalolycopine and acetylanhydro-

aposerratinine were identical.

Alopecurine Hydrobromide

Fuming hydrobromic acid in acetone (2%) was added in a dropwise fashion to alopecurine (5 mg) dissolved in methylene chloride until the resulting solution tested red on moist litmus paper. This solution was then allowed to evaporate at room temperature. The residual crystalline mass was recrystallized from methanol-acetone. The vacuum dried crystals decomposed at a temperature above 300°. The density of the crystals was determined by achieving neutral buoyancy for a crystal in a mixture of chlorobenzene ($d = 1.1 \text{ g/cm}^3$) and carbon tetrachloride ($d = 1.6 \text{ g/cm}^3$), and then measuring the density of the mixture. Found: $d^{25^\circ} = 1.35 \text{ g/cm}^3$ (± 0.01).

Alopecurine Hydrochloride

Anhydrous hydrogen chloride was briefly bubbled through a solution of alopecurine (5 mg) in methanol. Slow evaporation of the solution at room temperature gave a residual crystalline mass which was recrystallized from acetone. The crystals were dried under vacuum at room temperature and decomposed at a temperature above 290°.

The density of the crystals was determined in the same manner as for the hydrobromide. Found: $d^{25^\circ} = 1.257 \text{ g/cm}^3$ (± 0.002).

Alopecurine Methobromide

Alopecurine (10 mg) was dissolved in methanol and

refluxed with methyl iodide for 24 hr. The solution was evaporated and the residue dissolved in water. This aqueous solution of alopecurine methiodide* was washed with chloroform and then percolated through a column of Dowex 2-X8 (1 g). The column of Dowex resin had been prepared by washing with aqueous lithium bromide, the excess lithium bromide had been washed out with water. The alopecurine methobromide was eluted from the column with water and evaporated to give a crystalline mass (11 mg). Repeated recrystallization from methanol-acetone gave large crystals, mp 247-249°. Anal. Calcd for $C_{24}H_{23}BrNO_2$: C, 62.33; H, 6.97. Found: C, 62.26 and 62.44; H, 7.12 and 7.04%; ir (Nujol) 3300, 1725, 1595, 1580, 1260, and 1110 cm^{-1}; $d^{25^\circ} = 1.397\ g/cm^3$.

Hydrolysis of Alopecurine (80a)

Aqueous potassium hydroxide was added to a methanol solution of alopecurine (30 mg) and the solution was refluxed for 2 hr. The residue obtained by evaporating the solvent was dissolved in chloroform and washed with water. Evaporation of the dried ($MgSO_4$) chloroform layer gave crude crystals (16 mg) which were recrystallized from methanol-acetone, mp 230-232°. This compound was identical with the debenzoylalopecurine (87a) isolated from the plant. Calcd for $C_{16}H_{25}NO_2$: molecular weight 263.1885. Found: 263.1886 (mass spectrometry); ir (Nujol) 3350 cm^{-1}, no absorption

---

*Previously prepared by S. Valverde-Lopez, Ph.D. thesis, U. of A., 1966.

between 1800 and 1500 cm^{-1}.

Debenzoylalopecurine Hydrobromide (79a)

The hydrobromide was prepared in the usual way and crystallized from acetone-methanol, mp 249-251°. Anal. Calcd for $C_{16}H_{26}BrNO_2$: C, 55.81; H, 7.61. Found: C, 55.97 and 56.02; H, 7.43 and 7.53%; ir (Nujol) 3310 and 3460 cm^{-1}; d^{25° = 1.481 g/cm^3 ($\pm$ 0.002).

Acetylation of Alopecurine (80a)

Acetic anhydride (1 ml) was added to a pyridine solution (1 ml) of alopecurine (20 mg) and the solution was allowed to stand overnight. The solvent was then evaporated to leave an oil which could not be crystallized from acetone or ether; ir (CCl_4) 1735 and 1720 cm^{-1}, no OH absorption band; nmr ($CDCl_3$) τ 4.6 (1H, m), 4.8 (1H, d with fine structure, J = 8 cps), 7.9 (3H, s), 9.0 (3H, d, J = 6 cps), 1.9 and 2.5 (5H, aromatic).

Acetylalopecurine perchlorate was prepared in the usual way, mp over 300°. Anal. Calcd for $C_{25}H_{32}ClNO_8$: C, 58.87; H, 6.32%. Found: C, 59.59; H, 6.11%; ir (Nujol) 3175, 1725, 1700, 1595, 1580, 1500, 1275, 1250, and 1100 cm^{-1}.

Oxidation of Alopecurine (80a)

Jones' reagent (8 drops) was added to a solution of alopecurine (46 mg) in purified acetone (8 ml). Methanol (10 drops) was added and the solution evaporated to near dryness. The residue dissolved in chloroform was washed with dilute aqueous sodium bicarbonate. The dried chloroform solution ($MgSO_4$) was

evaporated to yield an oil (81a) (24 mg); ir ($CHCl_3$) 1715 (benzoate), 1690 (C=O), 1410 cm^{-1} ($-CH_2-CO-$), and no OH; tlc indicated the oil was homogeneous but crystallization could not be induced in various solvents. Molecular distillation (150°/0.1 mm) gave back the unchanged oil; nmr ($CDCl_3$) τ 4.7 (1H, m), 4.5 (1H, d, J = 9 cps), 4.65 (1H, q, J = 9 and 2 cps), 6.25 (1H, q, J = 16 and 6.5 cps), 7.55 (1H, q, J = 17 and 8.5 cps), 7.0 (1H, q, J = 16 and 1 cps), 8.0 (1H, q, J = 17 and 2.5 cps), 9.0 (3H, d, J = 6 cps), and τ 2-2.5 (5H, aromatic). Calcd for $C_{23}H_{27}NO_3$: molecular weight 365. Found: 365 (mass spectrometry).

Reduction of Dehydroalopecurine (81a)

Sodium borohydride (10 mg) was added to a methanol solution of dehydroalopecurine (8 mg) and allowed to stand overnight. The methanol was evaporated and the residue, dissolved in water, was extracted with chloroform. The dried ($MgSO_4$) chloroform extracts were evaporated and the residue taken up in a little acetone. On standing the acetone solution deposited crystals, mp 243-245°, unchanged on admixture with alopecurine. The ir (Nujol) spectrum was identical with that of alopecurine.

Dehydration of Alopecurine (80a)

Alopecurine (25 mg) was dissolved in benzene (10 ml) and thionyl chloride (0.5 ml) added. The solution was refluxed overnight. Water was added, the solution made basic with ammonium hydroxide and the benzene layer separated. The aqueous layer was extracted twice with benzene. The combined, dried ($MgSO_4$)

benzene fractions were evaporated to give an oil (20 mg). Chromatography of this oil over neutral alumina (1 g) and elution with ether gave a compound (82a) (homogeneous by tlc) which crystallized from ether, mp 105-107°; ir ($CHCl_3$) 1720, 1610, 1590, 1280, and 1110 cm^{-1}. Calcd for $C_{23}H_{27}NO_2$: molecular weight 349.2042. Found: 349.2045 (mass spectrometry); nmr ($CDCl_3$) τ 4.7 (1H, m), 4.5 (1H, d, J = 9 cps), 4.65 (1H, q, J = 9 and 2 cps), 6.25 (1H, q, J = 16 and 6.5 cps), 7.55 (1H, q, J = 17 and 8.5 cps), 7.0 (1H, q, J = 16 and 1 cps), 8.0 (1H, q, J = 17 and 2.5 cps), 9.0 (3H, d, J = 6 cps), and τ 2-2.5 (5H, aromatic).

Hydrolysis and Oxidation of Anhydroalopecurine (82a)

Anhydroalopecurine (10 mg) was hydrolyzed with potassium hydroxide in the usual way to give a tlc homogeneous oil (5 mg); ir ($CHCl_3$) 3600 and 1665 cm^{-1}. Jones' oxidation of this anhydrodebenzoylalopecurine (83a) gave another tlc homogeneous oil (1 mg); ir ($CHCl_3$) 1710 cm^{-1}. This compound (84a) was not further characterized due to lack of material.

Hydrolysis of Dehydroalopecurine (81a)

Aqueous potassium hydroxide (5 drops) was added to a dioxane solution of dehydroalopecurine (36 mg). After 12 hours at room temperature the solvent was evaporated almost to dryness, the residue dissolved in water, and extracted with chloroform. Evaporation of the dried ($MgSO_4$) chloroform solution gave a solid mass (85a) (21 mg) which was recrystallized from acetone, mp 160-162°. Calcd for $C_{16}H_{23}NO_2$: molecular weight 261.1728.

Found: 261.1729 (mass spectrometry); ir (Nujol) 1690 (C=O) and 1412 cm^{-1} ($-CH_2CO-$); uv max (MeOH) 225 mμ (ε 1,670); uv max (MeOH-HCl) 228 mμ (ε 800).

Oxidation of Debenzoylalopecurine (87a)

Jones' reagent (2 drops) was added to a purified acetone solution of debenzoylalopecurine (6 mg) at room temperature. Methanol (2 drops) was then added and the solution was evaporated. The residue was dissolved in chloroform and washed with dilute aqueous sodium bicarbonate. The dried ($MgSO_4$) chloroform layer was evaporated to give an oil (5 mg) which would not crystallize (86a). Calcd for $C_{16}H_{21}NO_2$: molecular weight 259.1572. Found: 259.1574 (mass spectrometry); ir ($CHCl_3$) 1720, 1690, 1430, 1410, and 1400 cm^{-1}. The hydrobromide of 86a was prepared in the normal way, mp 248-250° (dec.).

Oxidation of Debenzoyldehydroalopecurine (85a)

The oxidation was carried out with Jones' reagent in the normal way and the product obtained was identical with the diketone (86a) obtained in the oxidation of debenzoylalopecurine (87a).

Acetylation of Debenzoyldehydroalopecurine (85a)

Debenzoyldehydroalopecurine (5 mg) was acetylated with acetic anhydride-pyridine (1:1) in the usual way. The product was identical with the dehydroacetyldebenzoylalopecurine (88a) obtained in the following experiment.

Oxidation of Acetyldebenzoylalopecurine (89a)

The oxidation of acetyldebenzoylalopecurine (10 mg) with Jones' reagent (10 drops) was carried out in the usual manner. Evaporation of the dried ($MgSO_4$) chloroform extract gave a solid mass (9 mg) which was recrystallized from acetone, mp 241-243°. Calcd for $C_{18}H_{25}NO_3$: molecular weight 303.1834. Found: 303.1834 (mass spectrometry); ir (Nujol) 1750, 1695, and 1230 cm^{-1}.

Hydrolysis of Acetyldebenzoylalopecurine (89a)

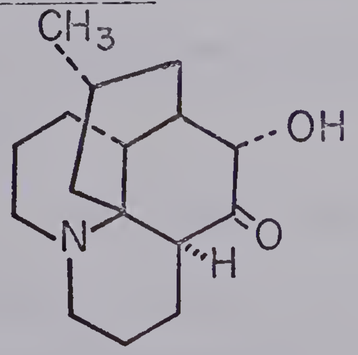
Hydrolysis of acetyldebenzoylalopecurine (18 mg) was carried out in methanol solution with potassium hydroxide at room temperature. After 24 hours it was worked up in the usual way. The diol (13 mg) obtained was identical with debenzoylalopecurine (87a).

DISCUSSION AND RESULTS

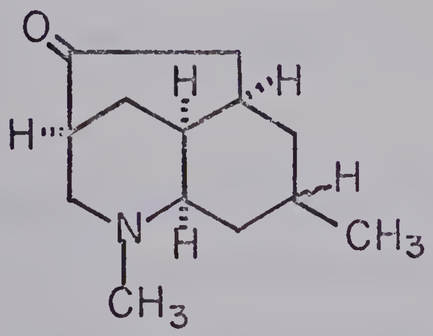
PART II

1. Isolation

The alkaloids of Lycopodium lucidulum Michx. were first investigated by Manske and Marion in 1946[70]. They reported the isolation of nicotine, lycopodine (2), alkaloid L.13 and six other bases which were designated alkaloids L.20, L.21, L.22, L.23, L.24, and L.25. Since that time the structure of a number of these alkaloids has been elucidated. Ayer and coworkers[71] have reported that the perchlorate of alkaloid L.13 is identical with lycopodine perchlorate. The structure of alkaloid L.20 (18) was was reported in 1963[29]. Ayer and coworkers[72] have suggested that alkaloid L.21 is the same as luciduline[10] (90), a weak base from L. lucidulum.



18



90

When the work reported in this thesis was started the structure of alkaloids L.22, L.23, L.24, and L.25 were unknown, and the availability of the plant prompted a further investigation of the alkaloids of this plant. Manske and Marion[70] had isolated and characterized these alkaloids as their perchlorate salts. They

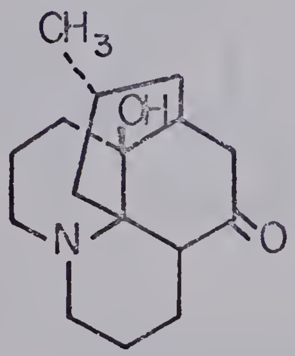
reported for alkaloid L.22, mp 108°, $C_{16}H_{27}NO$ (hydroperchlorate mp 254°); alkaloid L.23, mp 162°, $C_{16}H_{25}NO_2$ (hydroperchlorate mp 300°); alkaloid L.24, $C_{16}H_{25}NO$ (hydroperchlorate mp 278°); and alkaloid L.25, $C_{16}H_{25}NO_2$ (hydroperchlorate mp 297°).

Alkaloid L.23 has also been isolated from L. selago [73] and is identical with the pseudoselagine of Achmatowicz and Rodewald [74, 75].

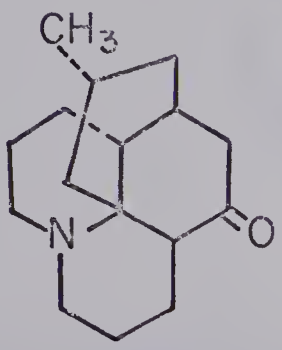
It has been known for some time that the presence of weak bases in the crude alkaloid extract of L. lucidulum complicates the chromatographic separation of the strong bases [76, 10]. For this reason the total crude alkaloid extract, obtained by the method reported by Berezowsky [76], was first separated into weak and strong bases according to the method developed by Nkunika [10]. An investigation of the "weak bases", initiated by D. Nkunika in 1963 is at present being conducted in these laboratories by L. F. Ball. Seven of the component alkaloids of the "strong bases" are reported in this thesis.

The "strong bases" of Lycopodium lucidulum Michx. obtained from the initial separation, were subjected to seven funnel countercurrent distribution between chloroform (moving phase) and potassium hydrogen phthalate buffer (pH 5). After completion of the distribution each fraction was made strongly basic and extracted into chloroform. Fraction 7, containing the weak bases was combined with the "weak base" fraction from the original separation. Each of the other six fractions were subjected to adsorption

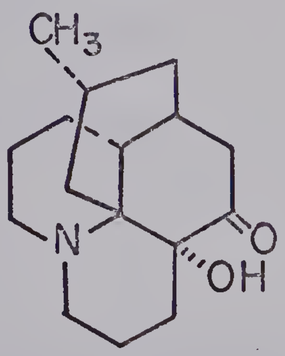
chromatography over alumina. Elution with solvents of increasing polarity gave components of varying purity which were further purified by preparative thin-layer chromatography and crystallization. In a typical chromatogram countercurrent distribution fraction 3 was chromatographed over alumina. Elution with benzene and benzene-ether gave lycopodine (2). Elution with ether gave a mixture of lycodoline (14), mp 180°, and flabelliformine (91), mp 212°. On recrystallization from acetone the mixture melted at 183-184°. The components were separated by preparative thin-layer chromatography and identified by comparison with authentic samples.



14



2

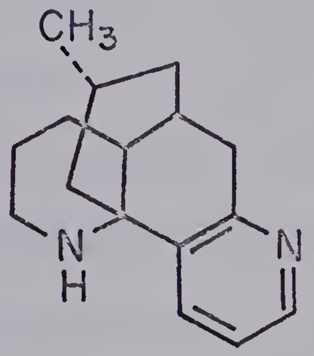


91

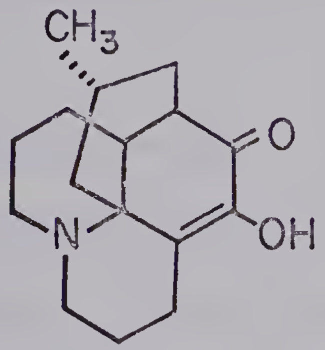
Flabelliformine (91) exhibits a great tendency to complex with other alkaloids. Berezowsky[76] has shown by mixing equimolar quantities of flabelliformine, mp 212°, with dihydrolycopodine (17), mp 168°, and lycopodine, mp 116°, in separate experiments, that 1:1 molecular complexes result with melting points 208° and 165° respectively.

Elution with ether-chloroform and chloroform gave after evaporation of the solvent mixed crystals of lycodoline (14) and

alkaloid L.23. These were separated by fractional crystallization from acetone. Lycodoline (14) crystallizes first leaving alkaloid L.23 in the mother liquor. Alkaloid L.23 was recrystallized from a small volume of acetone, mp 163-164° both alone and when admixed with an authentic sample of alkaloid L.23 provided by L. Marion [70]. The infrared spectra of the two compounds were superimposable. The structure of alkaloid L.23 will be discussed in Section 2 of this part of the thesis. Elution with chloroform-methanol (4:1) gave a diosphenol (92) which has previously been prepared from alkaloid L.20 [77].



5



92

Further quantities of alkaloid L.23 were obtained by the chromatography of countercurrent distribution fractions 2 and 4.

Chromatography of fraction 6 over alumina and elution with benzene-ether gave a mixture of lycopodine (2) and lycodine (5). These two compounds have very similar R_f values and can best be separated by reducing the mixture with sodium borohydride. The lycodine is unchanged under these conditions while the lycopodine is reduced to dihydrolycopodine (17). Dihydrolycopodine has a different R_f value compared to that of lycodine and the two compounds can be separated by chromatography over alumina.

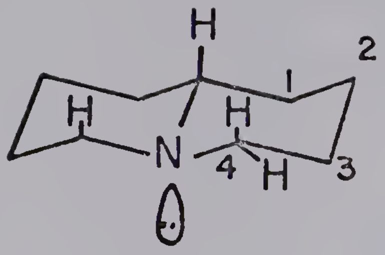
Further elution of fraction 6 with chloroform-methanol gave a compound, mp 230-233°, identical (mp, mmp, ir spectrum) with that previously isolated from L. clavatum var. megastachyon[78]. This compound has been given the name lucidioline and will be discussed in detail in section 3.

The molecular formula of lucidioline corresponds to that assigned to alkaloid L.25 by Manske and Marion[70]. However, comparison of the infrared spectra of a sample of alkaloid L.25 supplied by L. Marion and lucidioline showed that they were completely different. Alkaloid L.25 and alkaloid L.23 were found to be identical.

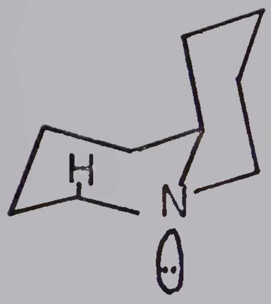
Alkaloid L.20 (18) was obtained from the "strong bases" by dissolving the crude alkaloids in methanol and allowing the solvent to evaporate slowly. Crystalline alkaloid L.20 was filtered off, washed and recrystallized from methanol-acetone, mp 258-259°. Alkaloid L.20 was not isolated in any of the chromatographies of the countercurrent distribution fractions.

2(a). Structure of Alkaloid L.23

In 1957 an important infrared criterion was reported by Bohlmann[79, 80] for distinguishing between trans-(93a) and cis-(94a) quinolizidine derivatives. Derivatives possessing a trans ring juncture with at least two axial hydrogen atoms on carbons adjacent to nitrogen show characteristic strong absorption bands in the 2800-2700 cm^{-1} region. Such a relationship is not possible in cis-quinolizidines and Bohlmann has shown that these compounds do

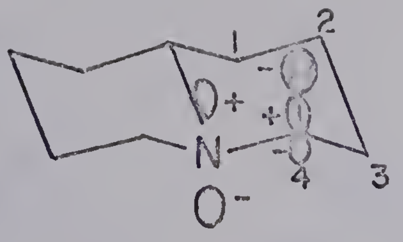


93a



94a

not show intense absorption in the 2700-2800 cm^{-1} region in their spectra. "Bohlmann bands" are not found in any amine salts, N-oxides or lactams. In more recent papers, the correlation of quinolizidine conformation with nuclear magnetic resonance spectra is reported [81, 82]. Hamlow and Okuda [81] have shown that in the nuclear magnetic resonance spectra of trans-quinolizidine (93a) the axial protons on the carbons adjacent to nitrogen appear at higher field than the corresponding equatorial protons. A chemical shift difference of 0.93 ppm of H_{ax} versus H_{eq} was observed for trans-quinolizidine, substantially larger than that observed in cyclohexane and sugar acetate derivatives [83]. When the nuclear magnetic resonance spectrum of trans-quinolizidine is determined in trifluoroacetic acid, the nitrogen lone pair is effectively bound and the chemical shift difference is only 0.5 ppm. The proposed rationalization for this phenomena is that in saturated cyclic nitrogen compounds in which the direction of the nitrogen lone pair is fixed, partial participation of the lone pair in an antibonding σ^* orbital between C-4 and the axial hydrogen takes place as shown in structure 95a. In other words the bond between C-4 and the nitrogen has some

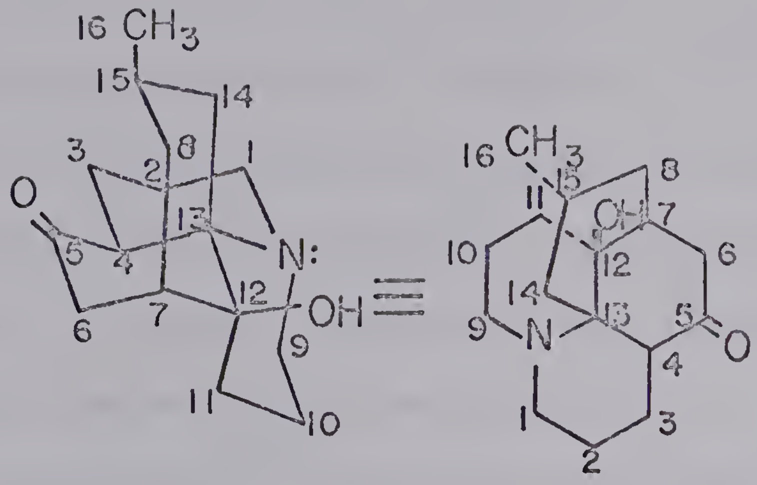


95 a

double bond character causing an increase in electron density at the C-4 axial proton. This would explain the shielding of this axial proton in the nuclear magnetic resonance spectrum and the low C-H stretching frequency in the infrared spectrum.

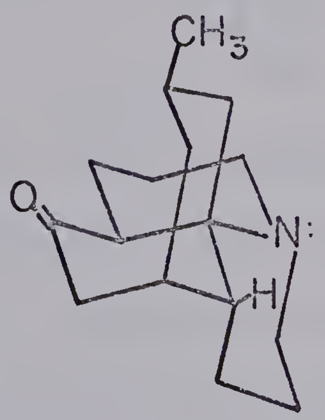
The infrared spectrum ($CHCl_3$) of alkaloid L.23 shows carbonyl absorption at 1690 cm^{-1}, a concentration independent band at 3510 cm^{-1} indicative of an intramolecularly hydrogen bonded hydroxyl, and intense "Bohlmann bands" at 2820, 2760, and 2680 cm^{-1} indicative of a trans-quinolizidine system. The nuclear magnetic resonance spectrum of alkaloid L.23 revealed the presence of the secondary C-methyl group (τ 9.03) and showed a one proton singlet at τ6.4. This signal disappeared when deuterium oxide was added to the sample and thus was assigned to the proton of an hydroxyl group. There were no other signals below τ 6.7 and this suggested that the hydroxyl group was tertiary. The finding that the alkaloid is resistant to acetylation is consistent with this observation.

The mass spectrum of alkaloid L.23 confirms the molecular formula $C_{16}H_{25}NO_2$ previously assigned[70] and is strikingly similar to that of lycodoline (14a).

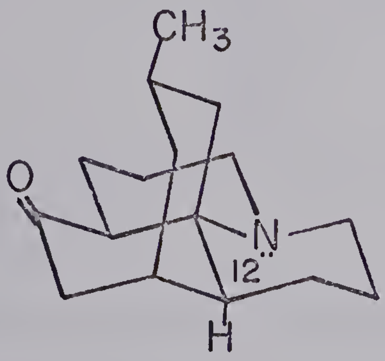

16 CH3
15
14
3
2
8
1
O
5
4
13
N:
12
OH
6
7
9
11
10
≡
16 CH3
15
8
OH
7
10
12
6
14
13
5
9
N
O
4
1
3
2

14 a 14

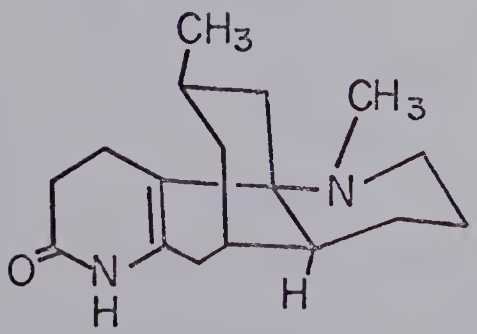
It has previously been shown that isomeric lycopodine alkaloids have similar mass spectra. The mass spectra of



2a

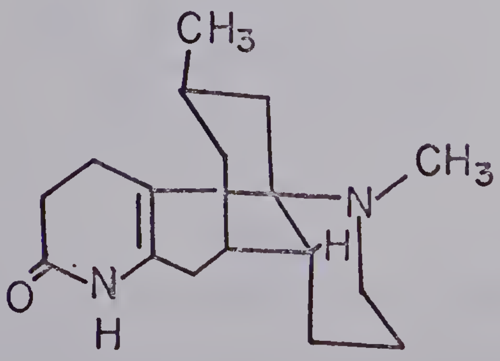


15a


CH3
CH3
N
O
N
H
H

Sauroxine

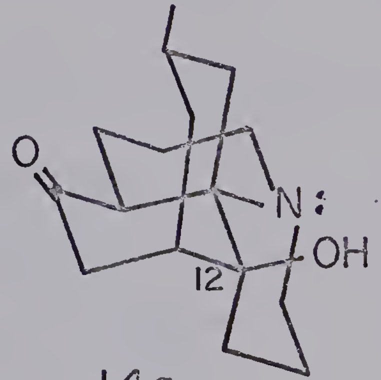
96 a


CH3
N
CH3
H
O
N
H

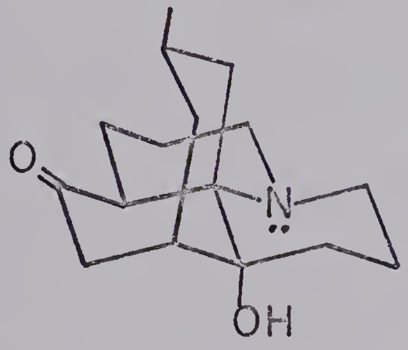
α -Obscurine

97a

lycopodine (2a) and epilycopodine (15a) are very similar as are the spectra of sauroxine (96a) and α-obscurine[84, 85] (97a). The similarity of the mass spectra of alkaloid L.23 and lycodoline (14a) suggest that they are stereoisomers. Since the infrared spectrum of alkaloid L.23 displays "Bohlmann bands" it must contain a *trans*-quinolizidine system as shown in structure 98a. Thus alkaloid L.23 and lycodoline (14a) are stereoisomeric at least at C-12 and nitrogen. This assignment of stereochemistry was consistent with the ultraviolet spectra of these compounds.

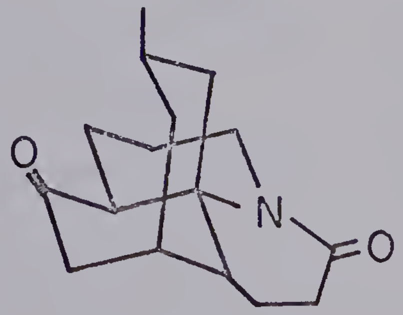


14a

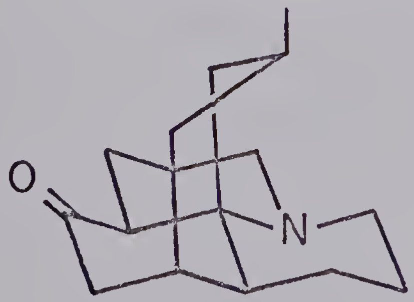


98a

The ultraviolet absorption bands in the region 215-230 mμ of a number of lycopodine type alkaloids are shown in table III.



99a



100a

The ultraviolet spectrum of lycodoline (14a) shows a maximum at 221 mμ (ε 1,500). This absorption band is not present in the spectra of salts of lycodoline and is also absent in the ultraviolet spectrum of alkaloid L.23, which shows only end absorption

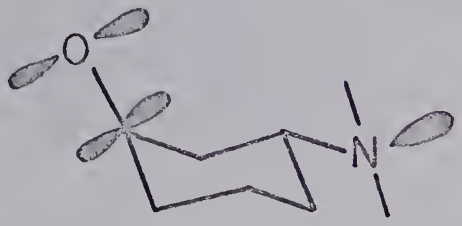
Table III

UV Spectra of Lycopodine Type Alkaloids in the Region 215-230 mμ

Compound	Solvent	λ_{max} (ε value)
Lycopodine (2a)	MeOH	217 mμ (2,400)$^+$
Lycopodine (2a)	Octane	217 mμ (2,400)$^+$
Alkaloid L.20 (18)	MeOH	220 mμ (2,500)$^+$
Lycodoline (14a)	MeOH	221 mμ (1,500)$^+$
Clavolonine (3)	MeOH	218 mμ (1,670)$^+$
Flabelliformine (91)	MeOH	228 mμ (inf) (≈500) $^+$
Epilycopodine (15a)	MeOH	No maximum
Lycopodine lactam (99a)	MeOH	No maximum
Alkaloid L.23 (98a)	MeOH	No maximum
Epiallolycopodine (100a)	MeOH	No maximum
Dihydrolycopodine (17)		No maximum

$^+$ Disappears on acidification of solution.

in this region. It has been shown by Cookson and coworkers[67] that β-aminoketones in which the unshared pair on nitrogen, the α, β σ-bond and the π orbital of the carbonyl are parallel (structure 101a illustrates the present case) give rise to a non-classical E.T. band in the 220-260 mμ region of the ultraviolet.

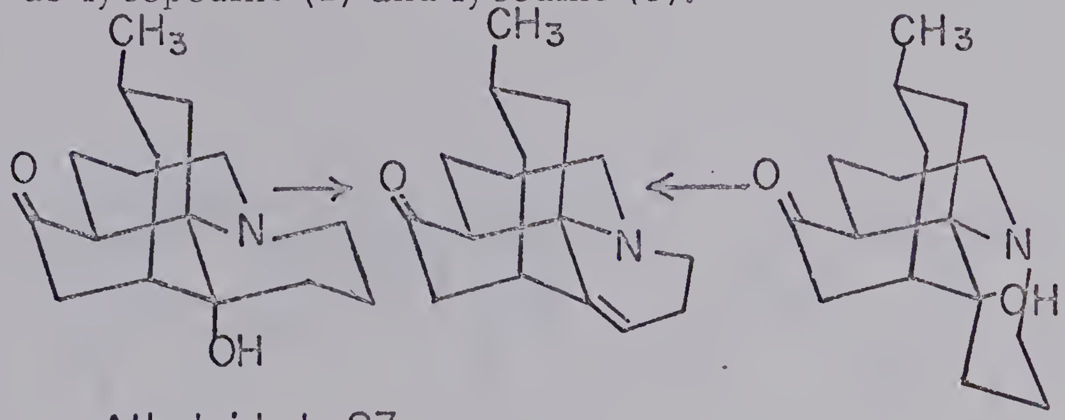


101 a

As may be seen in table III all of the lycopodine-like alkaloids with a carbonyl group at C-5 and in which the nitrogen lone pair is equatorial to ring A show an absorption band in this region whereas epilycopodine (15a) and alkaloid L.23 do not. Ultraviolet spectroscopy may therefore be used as an adjunct to infrared spectroscopy ("Bohlmann bands") in assigning the configuration at the nitrogen in these alkaloids. As will be discussed later, optical rotatory dispersion and/or circular dichroism may be used to the same end.

In order to establish that alkaloid L.23 differed from lycodoline (14a) only in the stereochemistry at C-12 and the nitrogen it was necessary to establish a direct correlation between the two. Dehydration of alkaloid L.23 with either phenylphosphonic dichloride in pyridine or with phosphorus pentoxide in toluene gave anhydrolycodoline (25a)[25, 44], isolated as its hydrochloride. Alkaloid L.23 is the second naturally occurring alkaloid of this group to possess the "epi" configuration at C-12, the other being sauroxine

(96a)[85]. Anhydrolycodoline has been transformed into lycopodine (2)[25] and thus alkaloid L.23 belongs to the same enantiomeric series as lycopodine (2) and lycodine (5).



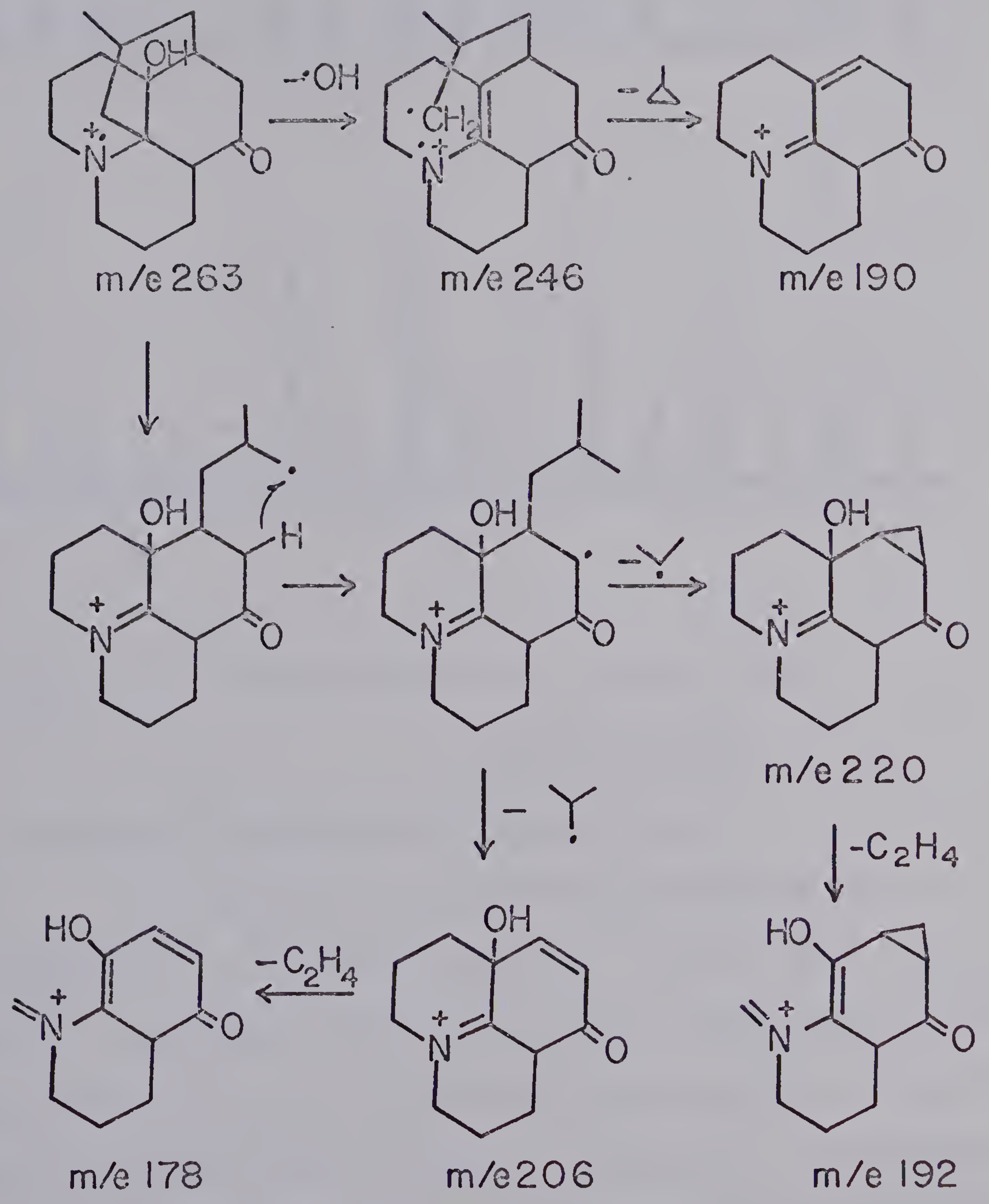
Alkaloid L.23

98a 25a (≐25) 14a

After this work had been completed Rodewald and Grynkiewicz[75] published structure 98a for pseudoselagine. This structural assignment was based mainly on the similarity of its mass spectrum to that of lycodoline. No direct correlation of the two compounds was reported.

Both alkaloid L.23 (98a) and lycodoline (14a) have a functional group instead of a hydrogen atom at C-12 and therefore might be expected to differ in their mass spectral fragmentation from lycopodine which was previously discussed. An examination of the spectra shown in figure 14 reveals a far more complex fragmentation than lycopodine. A rationalization of the fragmentation of alkaloid L.23 upon electron impact is shown in scheme VII.

Scheme VII


OH
N
O
-•OH
CH2
-C2H4
m/e 263
m/e 246
m/e 190
H
m/e 220
m/e 178
m/e 206
m/e 192
HO

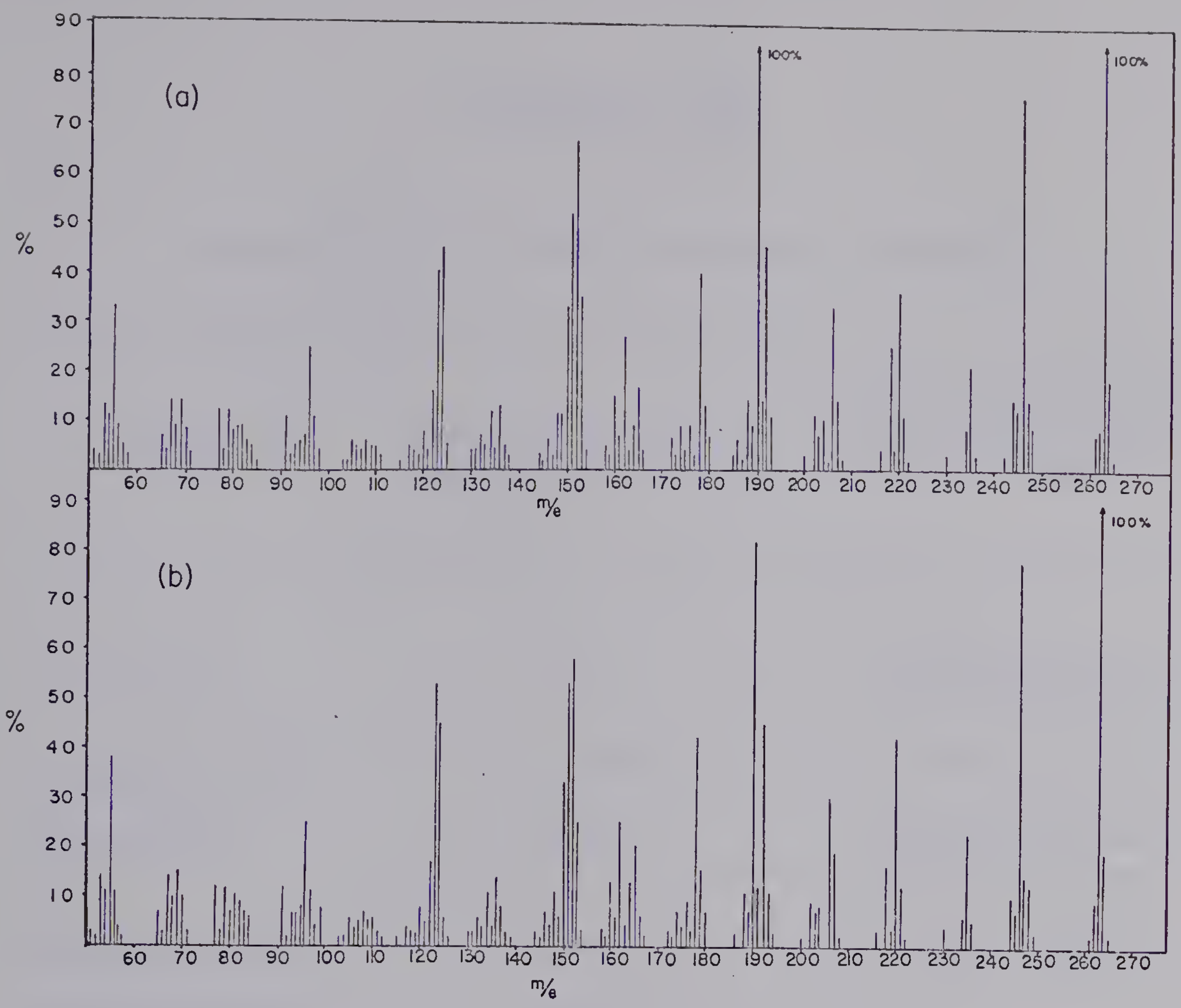


Figure 14 Mass Spectrum of (a) alkaloid L.23

(b) lycodoline

2(b). Absolute Configuration of Alkaloid L.23

The absolute configuration of lycopodine (2) was assigned by application [86, 46] of the octant rule [87] and the axial haloketone rule [29]. Lycopodine (2a) shows a positive Cotton effect (table IV) associated with the $n \rightarrow \pi^*$ transition of the keto group (figure 15a). This would predict that the absolute configuration of lycopodine should be as shown in 2a corresponding to the octant diagram 2b.

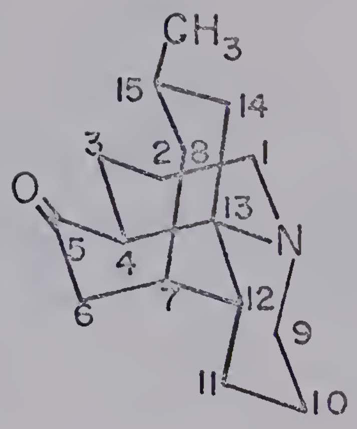
Table IV

Cotton Effects for Some Lycopodium Alkaloids

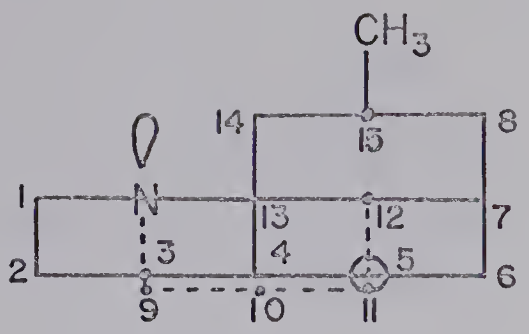
Compound	Rotatory Dispersion Molecular Amplitude*	Circular Dichroism Molecular Ellipticity (wavelength)
Lycopodine	203	11,700 (288 mμ) -16,200 (223 mμ)
Lycopodine·HCl	-28 !	-2,050 (290 mμ)
Lycodoline	198	10,900 (292 mμ) -16,350 (230 mμ)
Lycodoline·HCl	-39	-2,580 (288 mμ)
Epilycopodine**	-51 !	
Epilycopodine·HCl	-36 !	
Alkaloid L.23	-68	-4,230 (287 mμ)
Alkaloid L.23·HCl	-32	-2,470 (292 mμ)

* The symbol ! indicates that the extremum of shorter wavelength was not accurately measured.

** Values taken from reference 25.

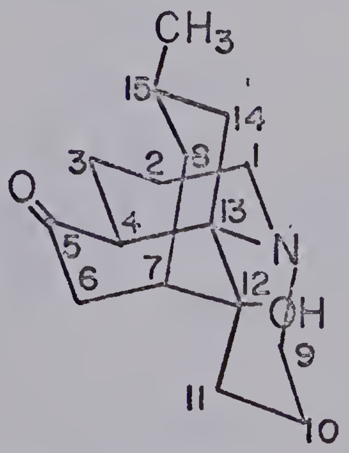

CH3
15
14
3
2
8
1
O
13
5
4
N
6
7
12
9
11
10

2a

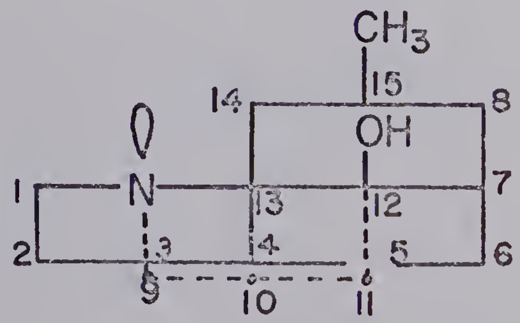

CH3
O
14
15
8
1
N
13
12
7
3
4
5
2
6
9
10
11

2b

Likewise lycodoline (14) shows a positive Cotton effect (figure 15c) and therefore should have the absolute configuration shown in 14a corresponding to the octant diagram 14b. This assignment of the same absolute configuration to both lycopodine (2a) and lycodoline (14a) is consistent with the fact that lycodoline (14a) has been converted into lycopodine (2a) by Ayer and Iverach[25].

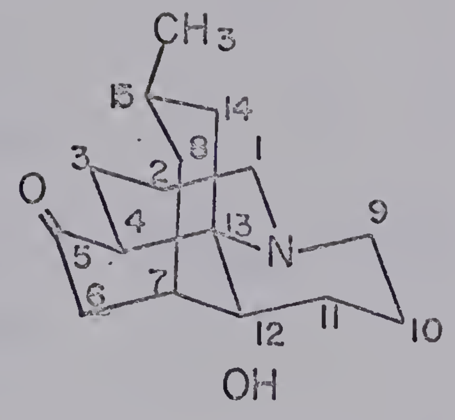

CH3
15
14
3
2
8
1
O
4
13
5
N
6
7
12
OH
9
11
10

14a

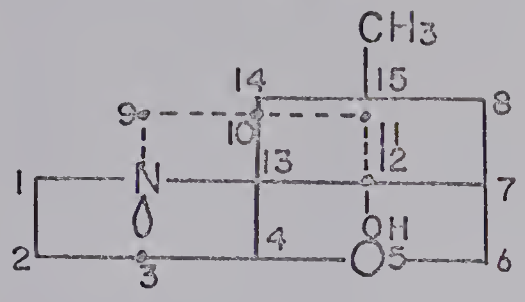

CH3
15
14
8
O
OH
1
N
7
13
12
2
3
14
5
6
9
10
11

14b

It has been shown above that alkaloid L.23 and lycopodine (2a) belong to the same enantiomeric series, therefore alkaloid L. 23 must have the absolute configuration shown in 40a. The octant diagram corresponding to 40a is shown in 40b. The octant rule predicts a positive Cotton effect for alkaloid L.23. Alkaloid L.23


CH3
15
14
3
2
8
1
O
4
13
9
5
N
6
7
12
11
10
OH

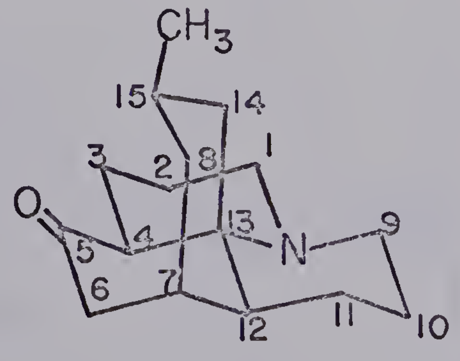
40a


CH3
14
15
9
8
10
11
13
12
1
N
7
O
OH
4
2
3
O5
6

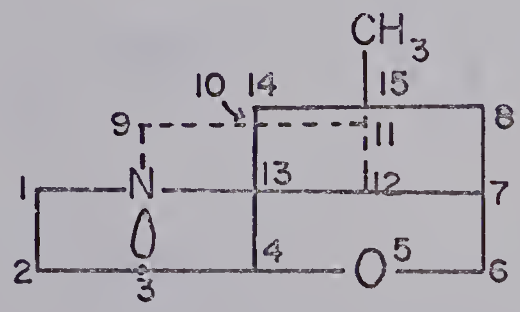
40b

however shows a negative Cotton effect (table IV) (figure 15c).

The salts of alkaloid L.23 also show a negative Cotton effect (figure 15f), although of lesser magnitude than that of the free base. In the case of lycopodine (2a) and lycodoline (14a), salt formation causes a reversal of the sign of the Cotton effect [29, 88] (figures 15b and 15d). Epilycopodine (15a = 15) (octant diagram 15b),


CH3
15
14
3
2
8
1
O
13
5
4
N
9
6
7
12
11
10

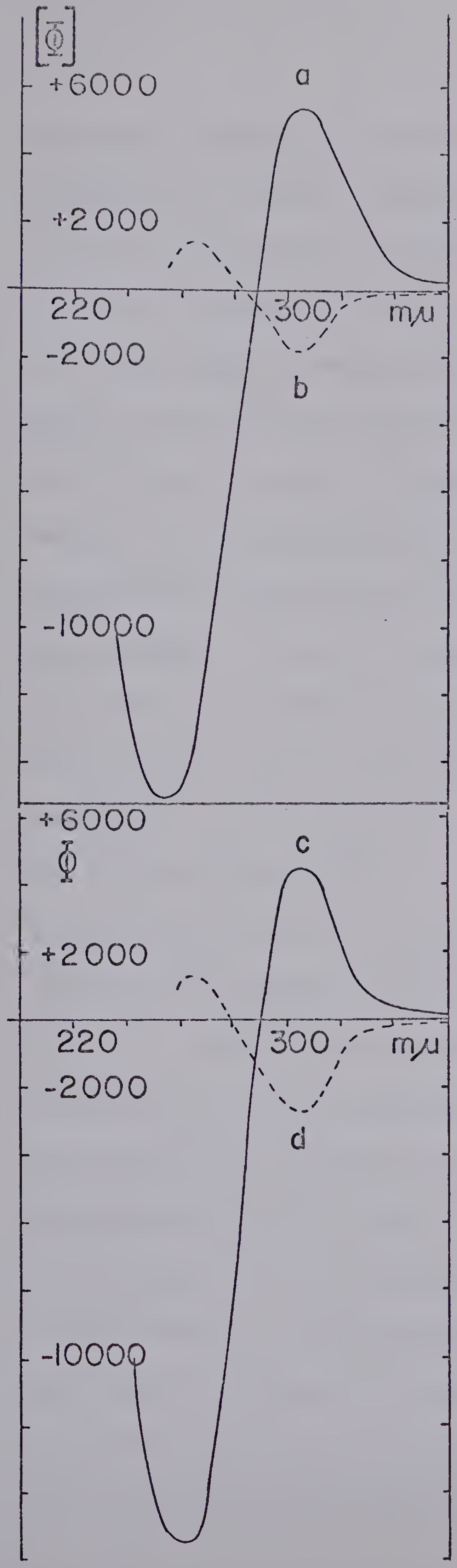
15 a


CH3
10
14
15
9
8
11
N
13
12
1
7
O
4
O5
2
3
6

15 b

like alkaloid L.23, shows a negative Cotton effect for both the salt and the free base (table IV). It appears therefore that the sign of the Cotton effect depends on the configuration at C-12 and nitrogen and only in the case of the free bases with the lycopodine configuration does the octant rule correctly predict the sign of the Cotton effect.

Circular dichroism measurements (table IV) show that



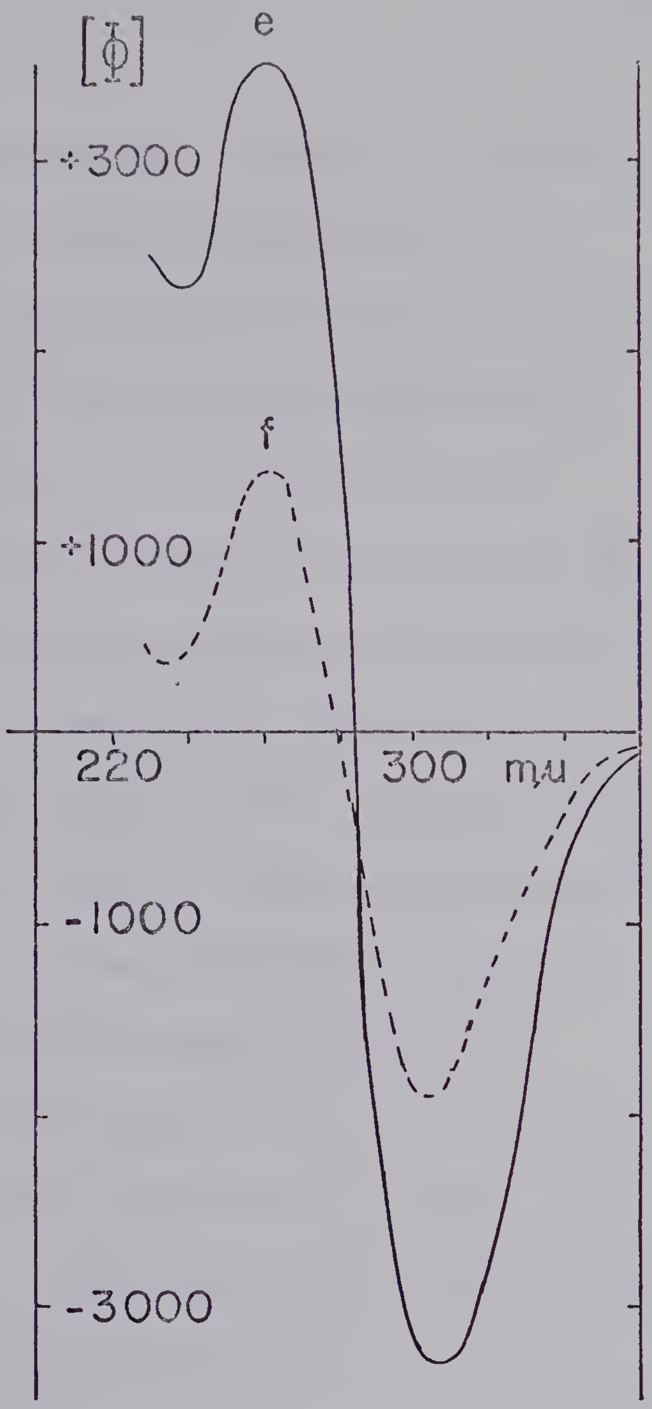


Figure 15

ORD spectrum of

(a) lycopodine

(b) lycopodine·HCl

(c) lycodoline

(d) lycodoline·HCl

(e) alkaloid L.23

(f) alkaloid L.23·HCl

lycopodine displays two Cotton effects above 200 mμ , a positive Cotton effect centered at 288 mμ associated with the n → π* transition and a negative Cotton effect centered at 223 mμ associated with the σ -coupled π interaction discussed above (figure 16a). The large negative rotation at 250 mμ (-15,000°) in the ORD (figure 15a) is thus the result of the overlapping of the troughs of the two Cotton curves. Protonation of the nitrogen removes the shorter wavelength Cotton effect in the CD curve and the one at longer wavelength becomes negative (figure 16b). The CD curve for alkaloid L.23 shows a single, negative, Cotton effect (figure 16c). It is thus obvious that the octant rule cannot be safely applied in these cases at least until the effect of nitrogen, charged and uncharged, is fully understood. The argument used previously in the case of lycopodine [29], i.e., that charged nitrogen makes an anti-octant contribution, does not explain the negative Cotton effect observed for alkaloid L.23 and epilycopodine.

It is now necessary to consider the possibility that lycopodine (2a) and not epilycopodine (15a) is the anomalous case and that the absolute configurational assignment should be reversed. Lycopodine (2a) is the system which shows the unusual charge transfer band and it is conceivable that the octant rule may not apply to such a system. The following observations lead to the belief that the originally assigned configuration is correct. Both 6α-bromolycopodine (102a) and its hydrobromide show Cotton effects which are much more positive than those of the corresponding unbrominated

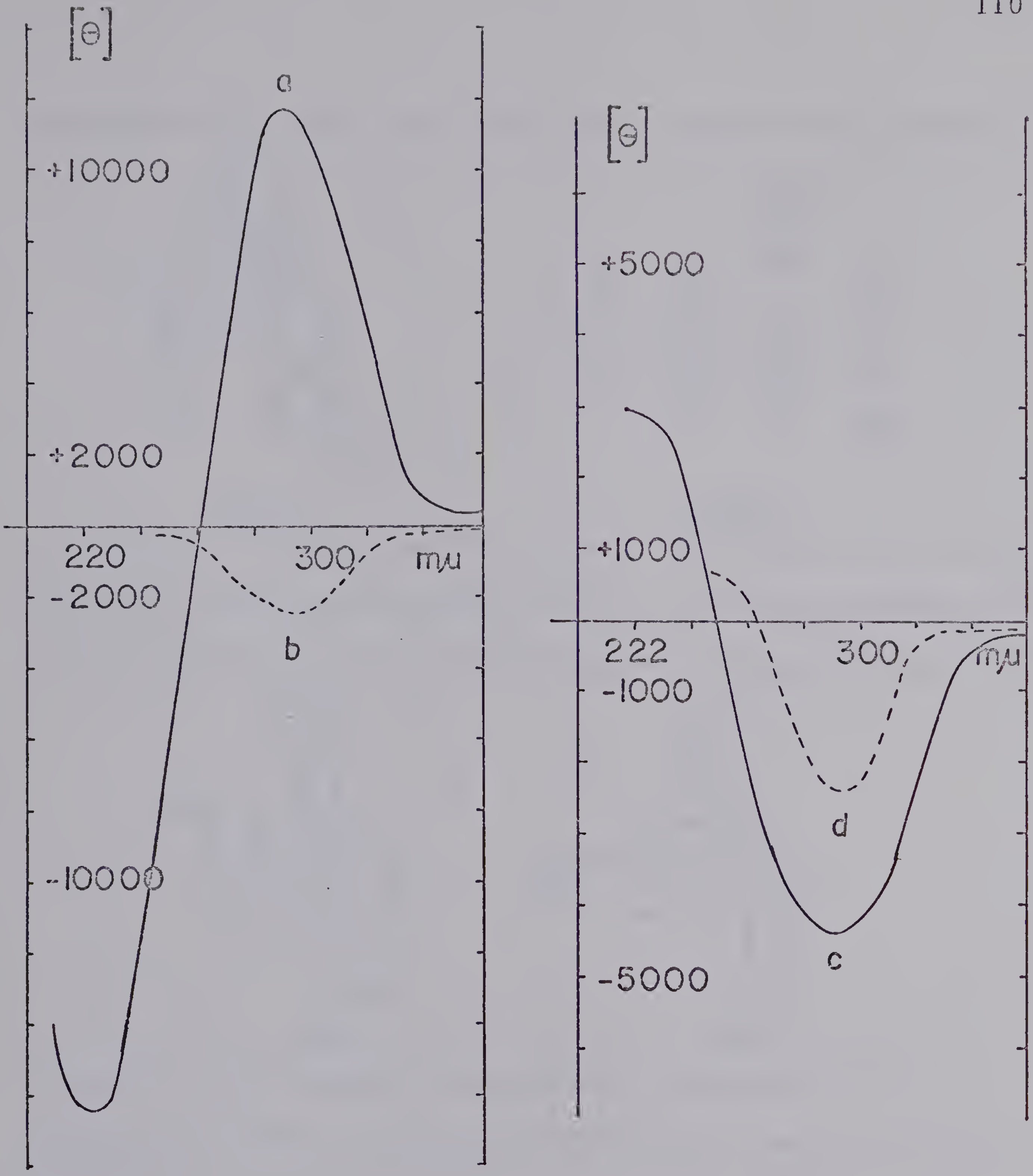


Figure 16

CD spectrum of

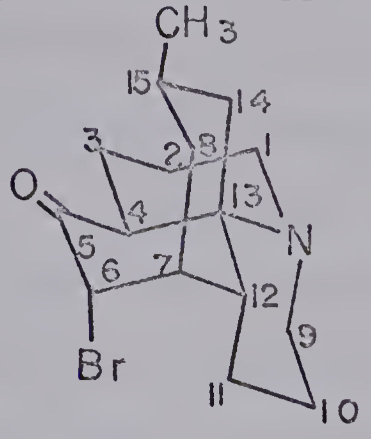
(a) lycopodine

(b) lycopodine·HCl

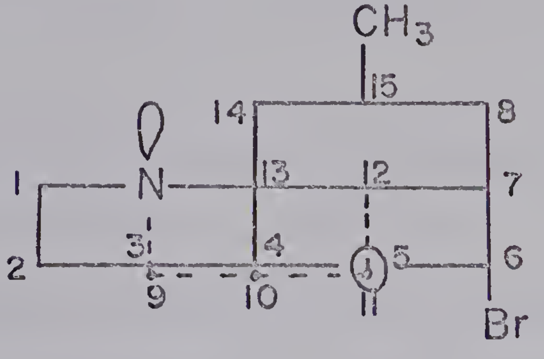
(c) alkaloid L.23

(d) alkaloid L.23·HCl

compounds [29]. Application of the axial haloketone rule should be

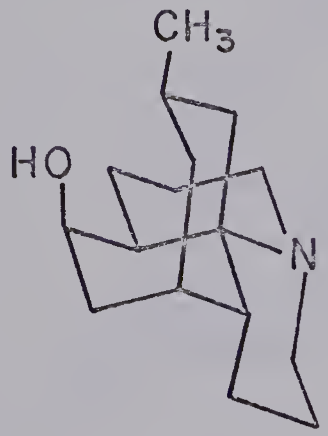

CH3
15
14
3
2
8
1
O
4
13
N
5
6
7
12
9
Br
11
10

102 a

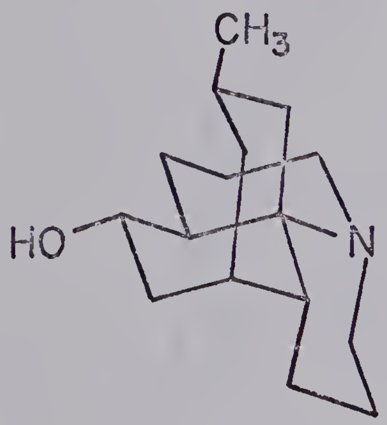

CH3
15
14
8
1
N
13
12
7
2
3
4
5
6
9
10
11
Br

102 b

independent of the effect of the nitrogen. Application of the Horeau method [89] to dihydrolycopodine (17a) and α-dihydrolycopodine (103a) gives results in agreement with the absolute configuration por-

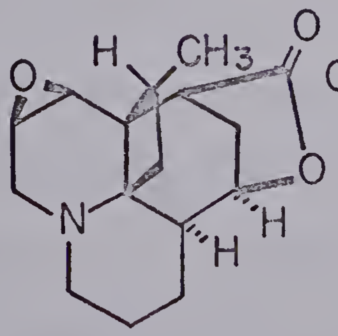


17a

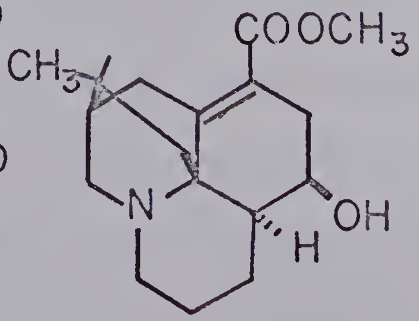


103 a

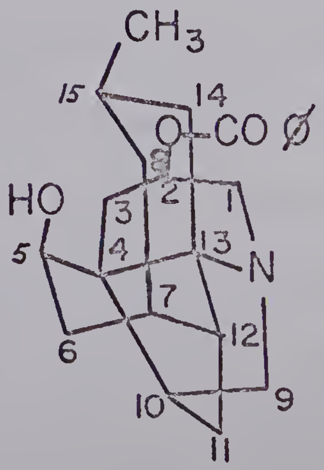
trayed [90]. The absolute configurations of annotinine [86] (1), annopodine [11] (104) and alopecurine (80a) have been determined,


O
H
CH3
O
O
N
H
H

1


COOCH3
CH3
N
OH
H

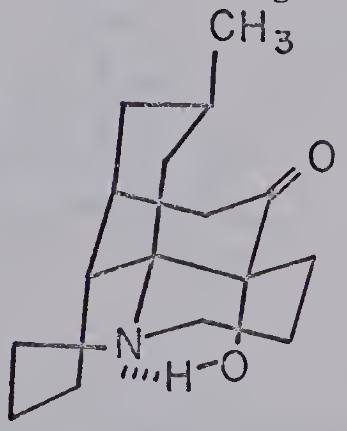
104


CH3
15
14
O-CO ϕ
HO
8
2
1
3
5
4
13
N
7
12
6
10
9
11

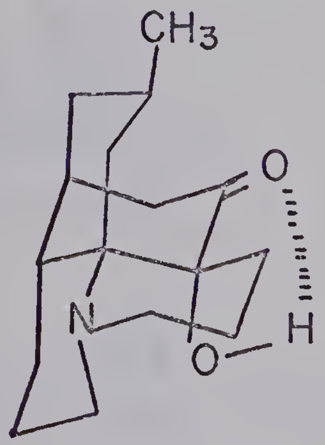
80 a

the latter two by the X-ray method, and although they have different skeletons they belong to the same absolute stereochemical series as that assigned to lycopodine.

It has been suggested by Curcumelli-Rodostamo and MacLean[91] that flabelliformine exists in conformation 91a rather than 91b since the hydroxyl group is intramolecularly hydrogen bonded (3560 cm^{-1} absorption in the infrared, not displaced on dilution) and it is inferred from the position of the carbonyl group (1705 cm^{-1}) in the infrared spectrum ($CHCl_3$) that this hydrogen bonding involves the nitrogen as receptor rather than the carbonyl.



91a

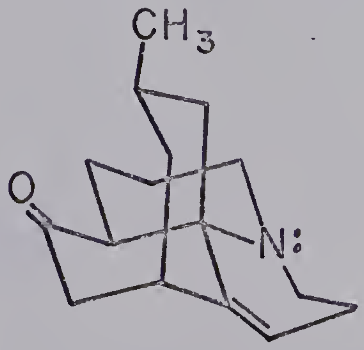


91b

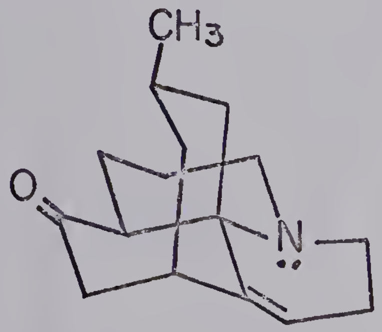
Since ORD, CD and uv measurements appear to offer a means of determining the orientation of the lone pair on the nitrogen in this system, the spectra of flabelliformine have been examined. The ultraviolet spectrum (table III) shows an inflexion at 228 mμ. A much clearer indication of the presence of this chromophore is obtained from the CD spectrum (MeOH) (figure 17a) which shows a strong ($\theta = -18,000$) negative Cotton effect at 225 mμ in addition to a positive Cotton effect ($\theta = +8,600$) at 293 mμ. The CD spectrum is very similar to that of lycopodine and lycodoline

(table IV). The ORD spectrum (figure 18a) (extrema 316 mμ (+2,250) and 257 mμ (-13,500°)) is also similar to that of lycopodine. Flabelliformine hydrochloride shows a negative Cotton effect (figures 17b and 18b). It thus appears that in methanol solution at least flabelliformine exists mainly in conformation 91b.

It has been reported[25] that anhydrolycodoline (25) also shows a strong positive Cotton effect (a = +13,300). This suggests that conformation 25a makes an important contribution to the description of the stereochemistry of anhydrolycodoline. Conformation 25b was previously assigned[25] on the basis of the "Bohlmann bands" present in the infrared spectrum. An explanation for these discrepancies may be that in both flabelliformine and anhydrolycodoline the infrared assignments were made on spectra determined in aprotic solvents and the ORD and CD measurements were made in protic solvents.



25 a



25 b

3. Structure of Lucidioline

The presence of a trisubstituted double bond in lucidioline, $C_{16}H_{25}NO_2$, was evident from the 1678 cm^{-1} band in the infrared spectrum and the one proton doublet at τ4.37 in the nuclear

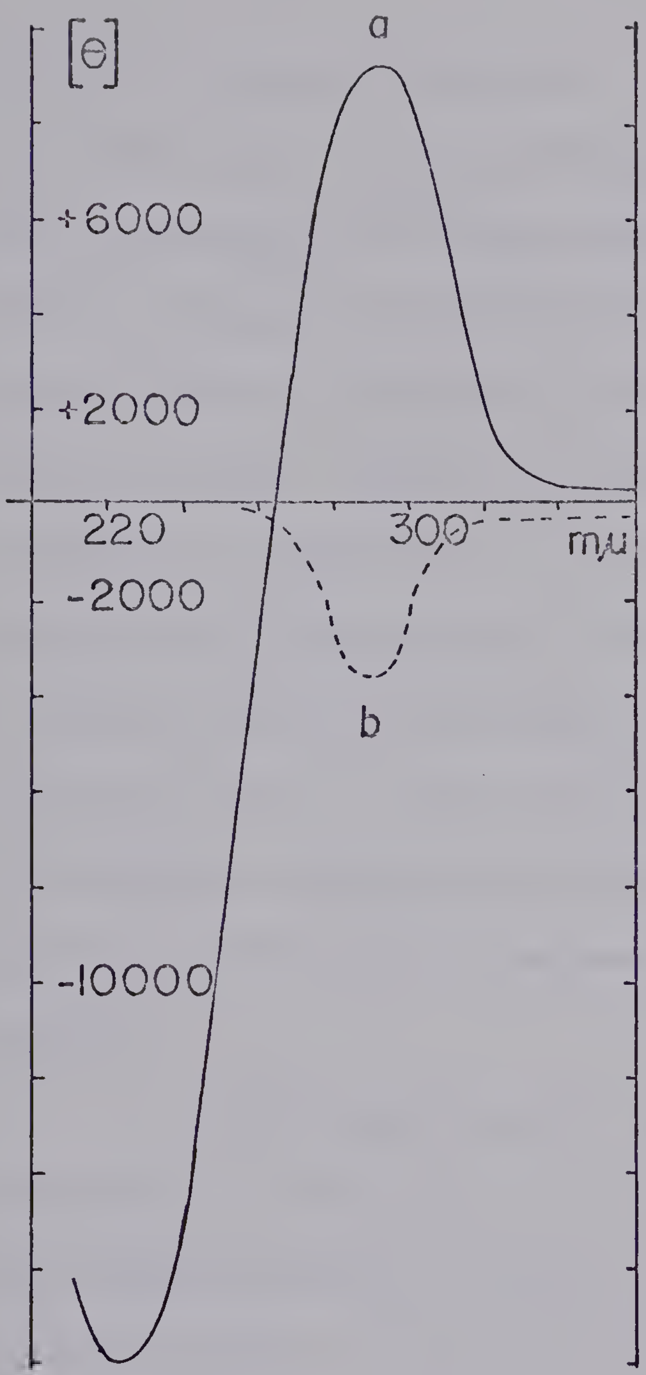


Figure 17

CD spectrum of

(a) flabelliformine

(b) flabelliformine·HCl

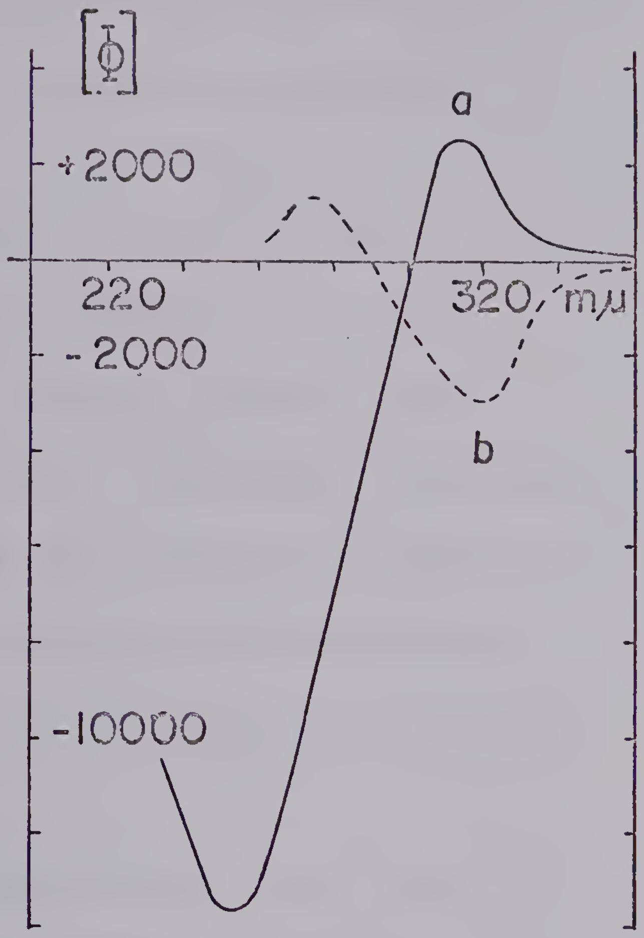


Figure 18

ORD spectrum of

(a) flabelliformine

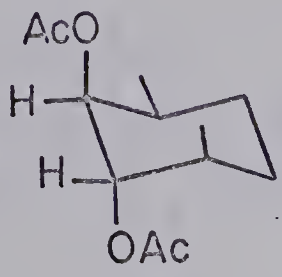
(b) flabelliformine·HCl

magnetic resonance spectrum. This was confirmed by hydrogenation over Adams' catalyst in methanol solution which resulted in the consumption of one mole of hydrogen to give predominantly the dihydro compound, mp 247-251°. Acetylation of lucidioline in acetic anhydride and pyridine gave a mixture of products which could not be purified. However, the dihydro compound under the same acetylating conditions gave O,O-diacetyldihydro compound, $C_{20}H_{31}NO_4$, which showed no OH or NH absorption, strong "Bohlmann bands" and a strong 1735 cm^{-1} absorption in the infrared spectrum (CCl_4). This result indicates that the two oxygen atoms in lucidioline are present as hydroxyl groups, the nitrogen is tertiary and the dihydro compound contains a trans-quinolizidine system.

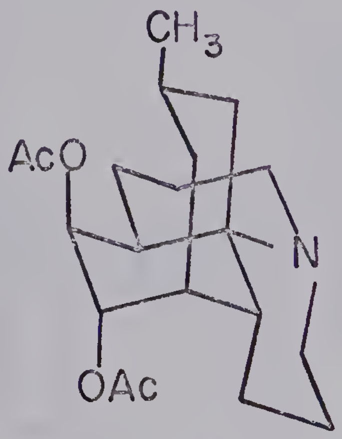
The mass spectrum of lucidioline proved to be very informative. The base peak in the spectrum at m/e 203 corresponds to the ion $C_{14}H_{21}N^{\dot{+}}$, as shown by high resolution measurements. An intense metastable peak centered at 156.8 indicates that the ion m/e 203 is formed directly from the molecular ion (calcd for 263 → 203; 156.5). This corresponds to the loss of $C_2H_4O_2$ and indicates that the two hydroxyl groups are on adjacent carbon atoms.

The configuration of these two adjacent hydroxyl groups was evident from an examination of the nuclear magnetic resonance spectrum of the O,O-diacetyldihydro compound. This spectrum has a doublet at τ5.06 (J = 7 cps) and a broad singlet at τ5.45

($W_{1/2}$ = 3 cps) and is consistent with a structure in which the hydrogens geminal to each acetoxy group are equatorial as shown in structure 105a. The same splitting pattern is present in acetyllycoclavine (106a) which is known to have two axial acetoxy groups[78].

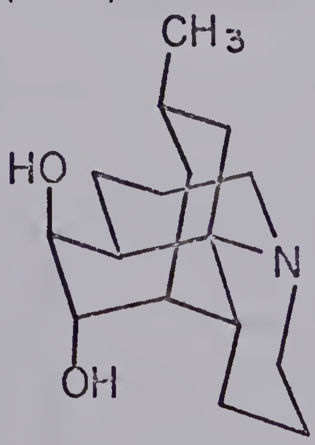


105a

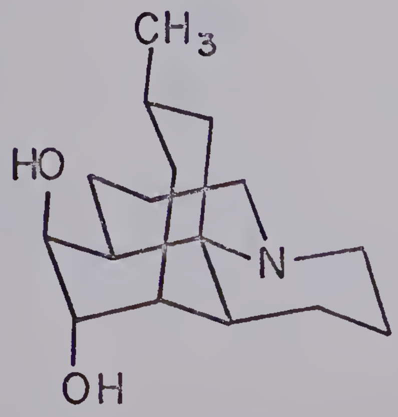


106 a

The mass spectrum of the dihydro compound, mp 247-251°, was very similar to the spectrum of desacetyllycoclavine (107a), mp 207-208° [78]. This observation together with the fact that the O,O-diacetyldihydro compound shows "Bohlmann bands" led to the postulate that the dihydro compound was 12-*epi*-desacetyllycoclavine (108a).



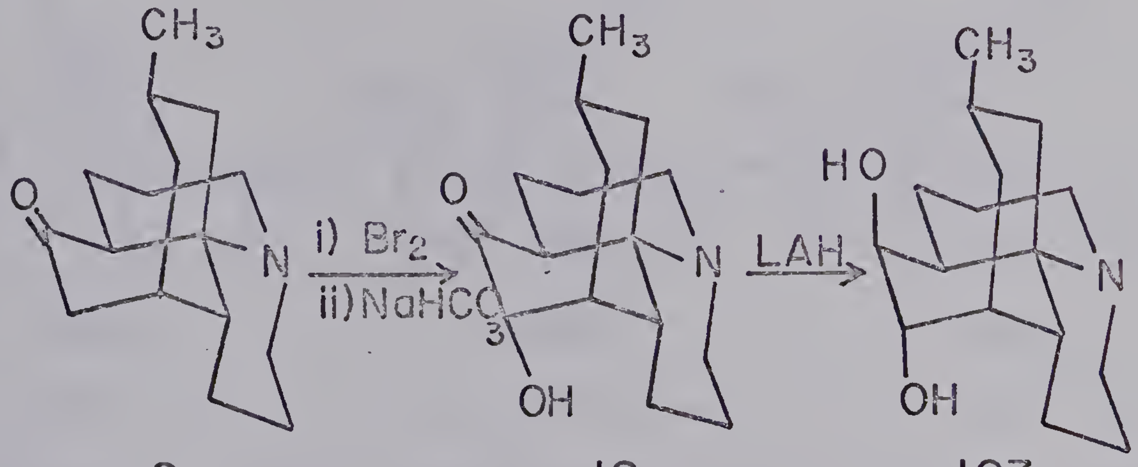
107a



108 a

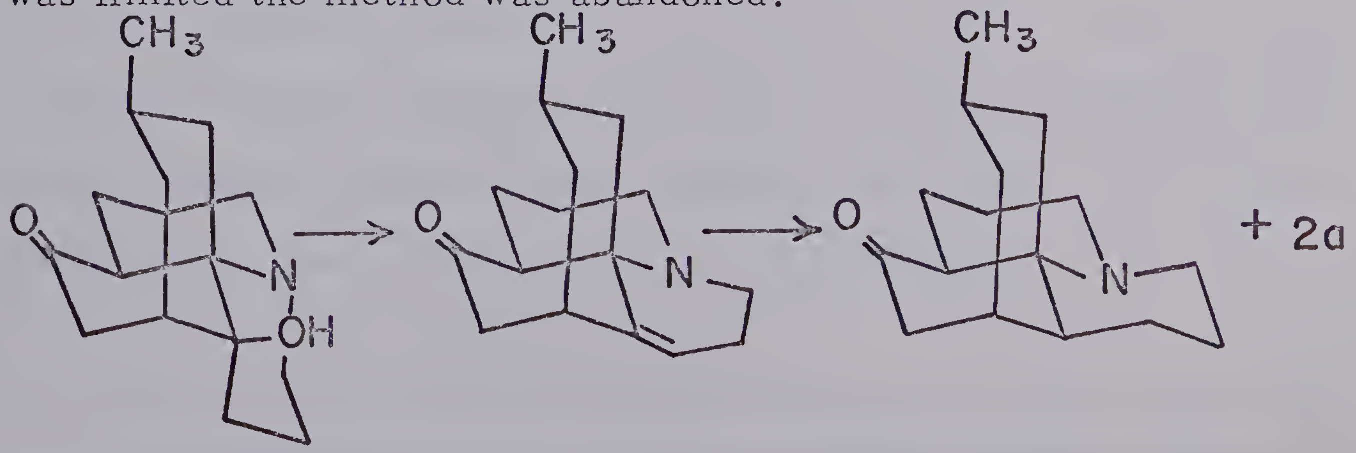
In order to prove this postulate it was decided to synthesize 12-*epi*-desacetyllycoclavine (108a). As was previously

noted, lycopodine (2a) has been converted into alkaloid L.20 (18a) and alkaloid L.20 reduced with lithium aluminum hydride to give desacetyllycoclavine (107a)[29]. A similar reaction sequence starting with epilycopodine (15a) should lead to 12-*epi*-desacetyllycoclavine (108a). Epilycopodine (15a) is not a naturally occurring



2a 18a 107a

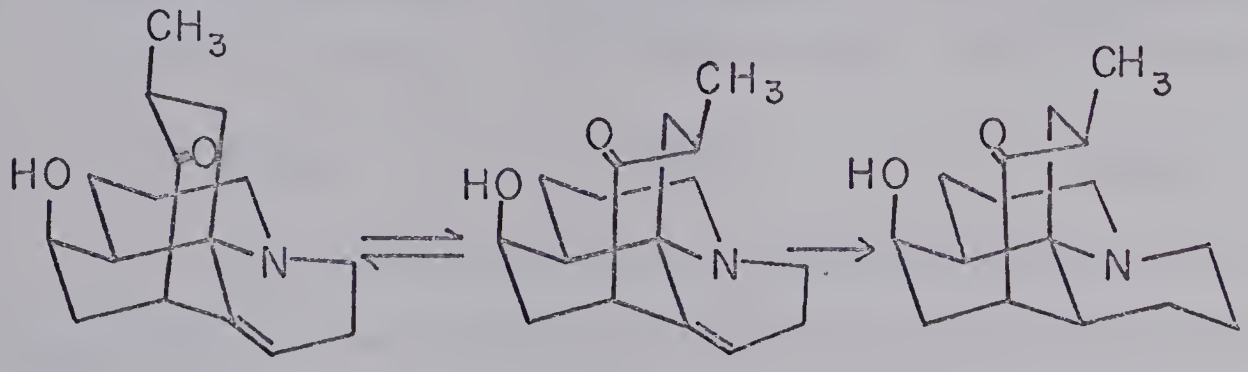
alkaloid and is obtained by synthesis from other suitably substituted alkaloids. Ayer and Iverach[25] prepared epilycopodine (15a) from lycodoline (14a) in 50% yield by the sequence (14a → 25a → 15a + 2a). However, application of this reaction sequence gave small yields of epilycopodine (15a) (10-20%) and because the supply of lycodoline was limited the method was abandoned.



14a 25a 15a

A reasonable supply (1.8 g) of acrifoline (36b = 36) was available and so synthetic routes to epilycopodine starting from this

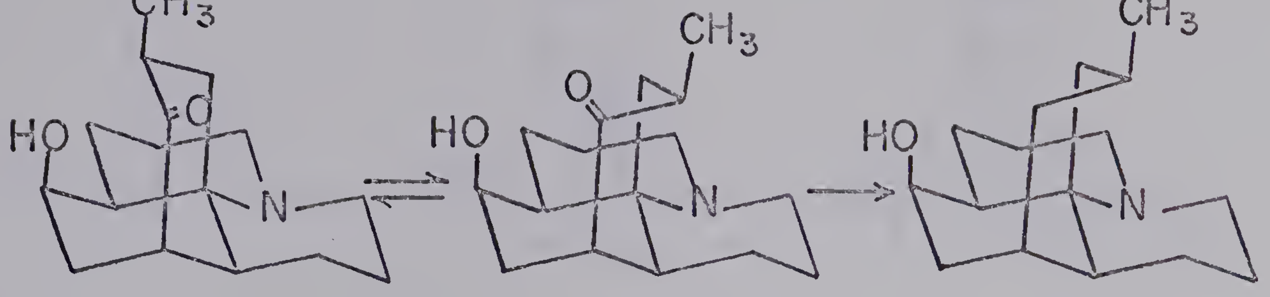
alkaloid were investigated. Anet[92] obtained a 90% yield of dihydroacrifoline (109a) by catalytic hydrogenation of acrifoline (36b). Dihydroacrifoline (109a) has the same stereochemistry at C-12 as epilycopodine (15a) and so this transformation was carried out. The next problem was to remove the keto group at C-8. Anet[92] has shown that under equilibrating conditions the boat form of acrifoline (36b) is in equilibrium with the chair form 36a and it is reasonable to expect that the same equilibrium exists in dihydroacrifoline (109a $\rightleftharpoons$ 109b). The main product from the Barton modification[93] of the Wolff-Kishner reduction of dihydroacrifoline (109a) was dihydroepiallolycopodine* (110a) possessing the unnatural and undesired configuration of the methyl group at C-15. The structure of dihydroepiallolycopodine (110a) was established by oxidation with Jones' reagent to give epiallolycopodine (111a) which was different from both lycopodine (2a) and epilycopodine (15a).



36a 36b 109a

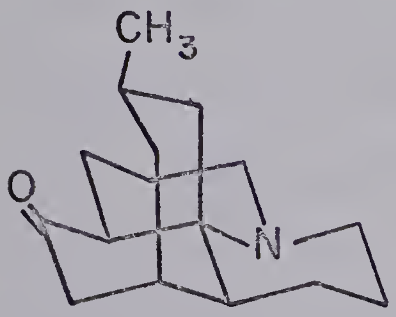
---

* Epiallolycopodine is the name of 12-*epi*-15-*allo*-lycopodine (110a).

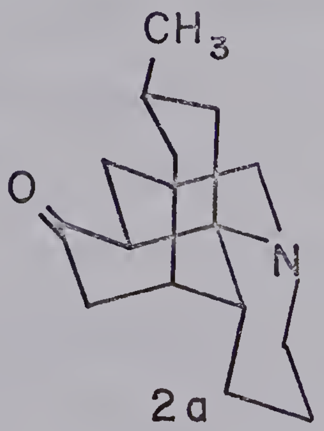


109b 109a 110a

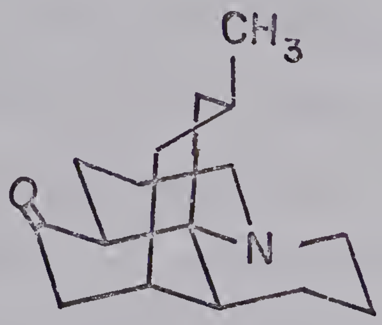
The molecular formula of epiallolycopodine (111a), $C_{16}H_{25}NO$, was confirmed by high resolution mass spectrometry. The mass spectra of these three isomers (2a, 15a and 111a) are all very similar. The infrared spectrum of epiallolycopodine displays "Bohlmann bands" and a carbonyl at 1700 cm^{-1}. The chemical shift of the methyl group in the nuclear magnetic resonance spectrum of epiallolycopodine is at τ 9.20, further upfield than the methyl group in either lycopodine (2a) or epilycopodine (15a) both of which occur at τ 9.14. This is what would be predicted from inspection of molecular models. The methyl group is more shielded by the carbonyl group in 111a than in either 2a or 15a.



15a

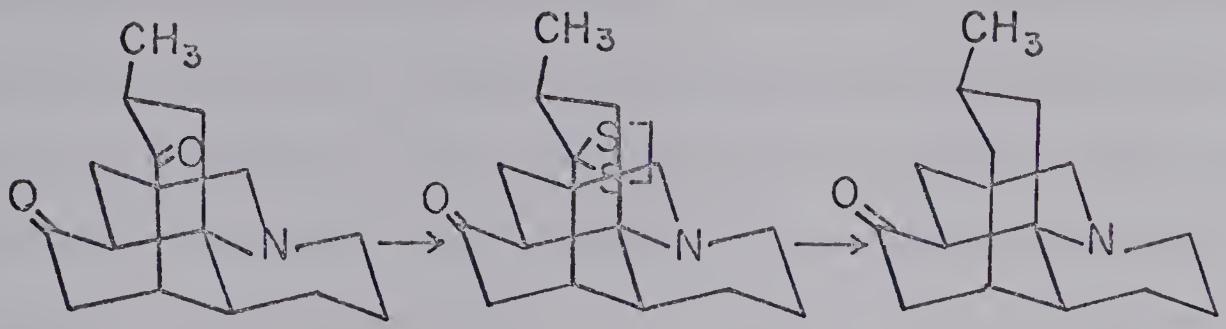


2a



111a

Oppenauer oxidation [94] of dihydroacrifoline (109a) gave a diketone which Iverach [95] has shown to have the chair form 112a.

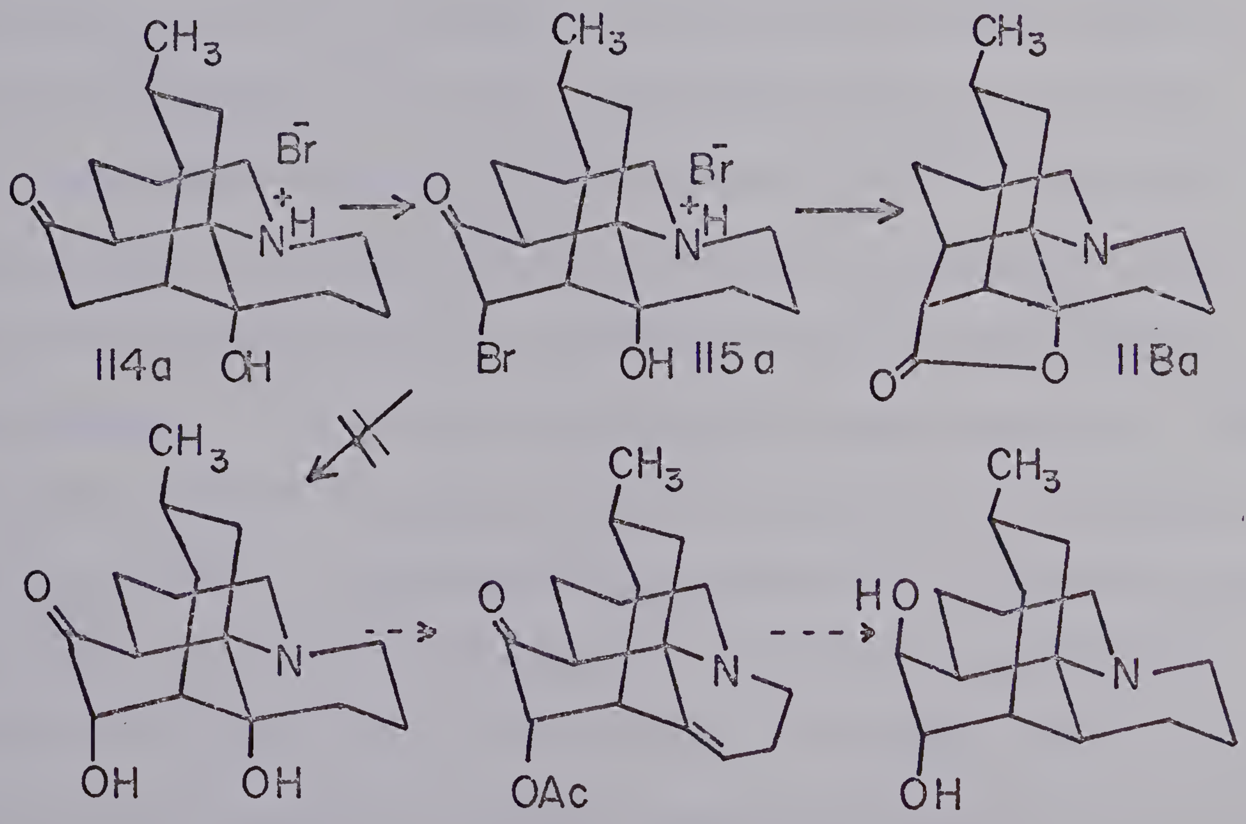


112a 113a 15a

The transformation of C-5 from a tetrahedral to a trigonal arrangement (oxidation of the hydroxyl to the ketone) removes the axial hydroxyl - C(15) interaction. The methyl group at C-15 can epimerize under the basic conditions of the Oppenauer reaction leading to the diketone 112a with ring D in a chair conformation. Thioketalization of 112a at room temperature gave a mixture of products. This mixture was not purified but desulfurized with Raney nickel. The Raney nickel (W-2) also partially reduced the carbonyl group at C-5, so in order to facilitate the chromatographic separation of this reduced mixture it was oxidized with Jones' reagent. In the thioketalization a reaction time of 36 hours was found to give the best yield of the monoethylenethioketal (113a) as indicated by the highest yield of epilycopodine in the final workup. Even under these optimum conditions the amount of epilycopodine was only 12% of the starting diketone 112a. This was insufficient to continue with the proposed synthesis of 12-epi-desacetyllycoclavine (108a).

A different approach to the synthesis of 12-epi-desacetyl-

lycoclavine (108a) was now attempted. Alkaloid L.23 hydrobromide (114a) was brominated with phenyltrimethylammonium perbromide to give a compound to which structure 115a is assigned. This labile bromination compound was not purified but immediately dissolved in aqueous sodium bicarbonate. The structure 115a is assigned to this compound by analogy to the bromination of lycopodine which is known to give 6α-bromolycopodine [29] (102a). Compound 115a was stirred in aqueous sodium bicarbonate in an attempt to obtain 6α-hydroxy-12-*epi*-lycodoline (116a). Compound 116a can hypothetically be converted into 12-*epi*-desacetyllycoclavine (108a) by way of compound 117a.



114a 115a 118a

116a 117a 108a

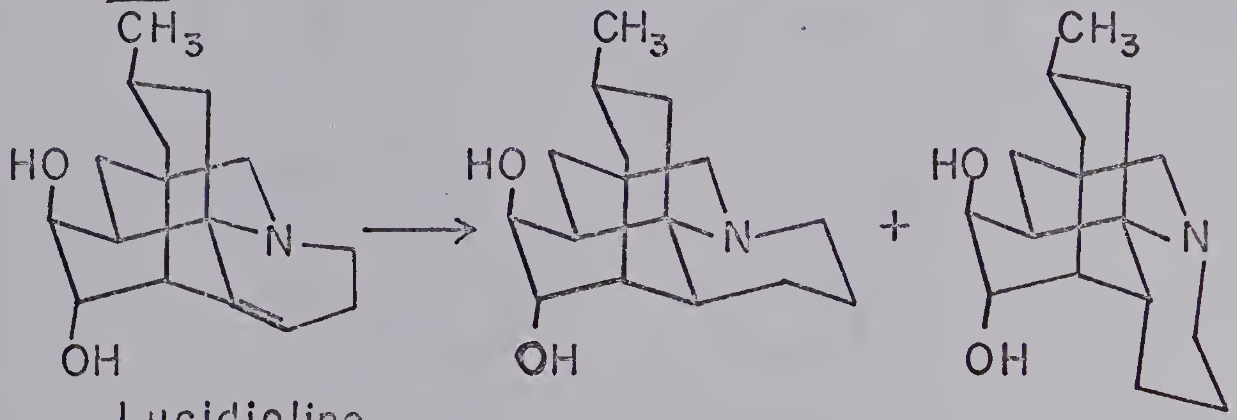
However, when compound 115a was stirred in aqueous sodium bicarbonate for 12 hours, compound 116a was not obtained,

instead a compound $C_{16}H_{23}NO_2$, mp 154-156°, ir (CCl_4) 1780 cm^{-1} was obtained which is tentatively assigned structure 118a. This compound may be formed by a Favorski rearrangement of the α-bromo-ketone 115a to give an acid which can lactonize to form 118a.

At this stage the synthesis of 12-epi-desacetyllycoclavine (108a) was abandoned and the structural elucidation of lucidioline approached in a different way.

As was noted above hydrogenation of lucidioline over platinum in methanol gave a product which consisted of two components. The minor component could not be isolated in pure form and was initially thought to be unreacted starting material (similar R_f), but careful examination on thin-layer chromatography showed it to be indistinguishable from desacetyllycoclavine (107a)[78]. When the hydrogenation was carried out in the presence of perchloric acid, the same two components were obtained but now in approximately equal amounts. The two were separated by chromatography. The component of higher R_f value proved to be identical with desacetyllycoclavine (107a), establishing that lucidioline is a dehydrodesacetyllycoclavine. Since the double bond in lucidioline is trisubstituted and since the methyl group is not located on the double bond (chemical shift in nuclear magnetic resonance spectrum), only the 11,12- or the 3,4-positions are possible for the double bond. The second product formed in the hydrogenation is isomeric with desacetyllycoclavine (107a). As was previously noted, acetylation gave an

O,O-diacetylderivative with prominent "Bohlmann bands" in the infrared spectrum, indicative of the presence of a trans-quinolizidine system in this compound. This indicates that the dihydro compounds are epimeric at C-12 (and at nitrogen) and identifies this carbon as one terminus of the double bond. Lucidioline thus has structure 119a and the two hydrogenation products are desacetyllycoclavine (107a) and 12-epi-desacetyllycoclavine (108a).



Lucidioline
119a

108a

107a

The mass spectrum of lucidioline (figure 19) is dominated

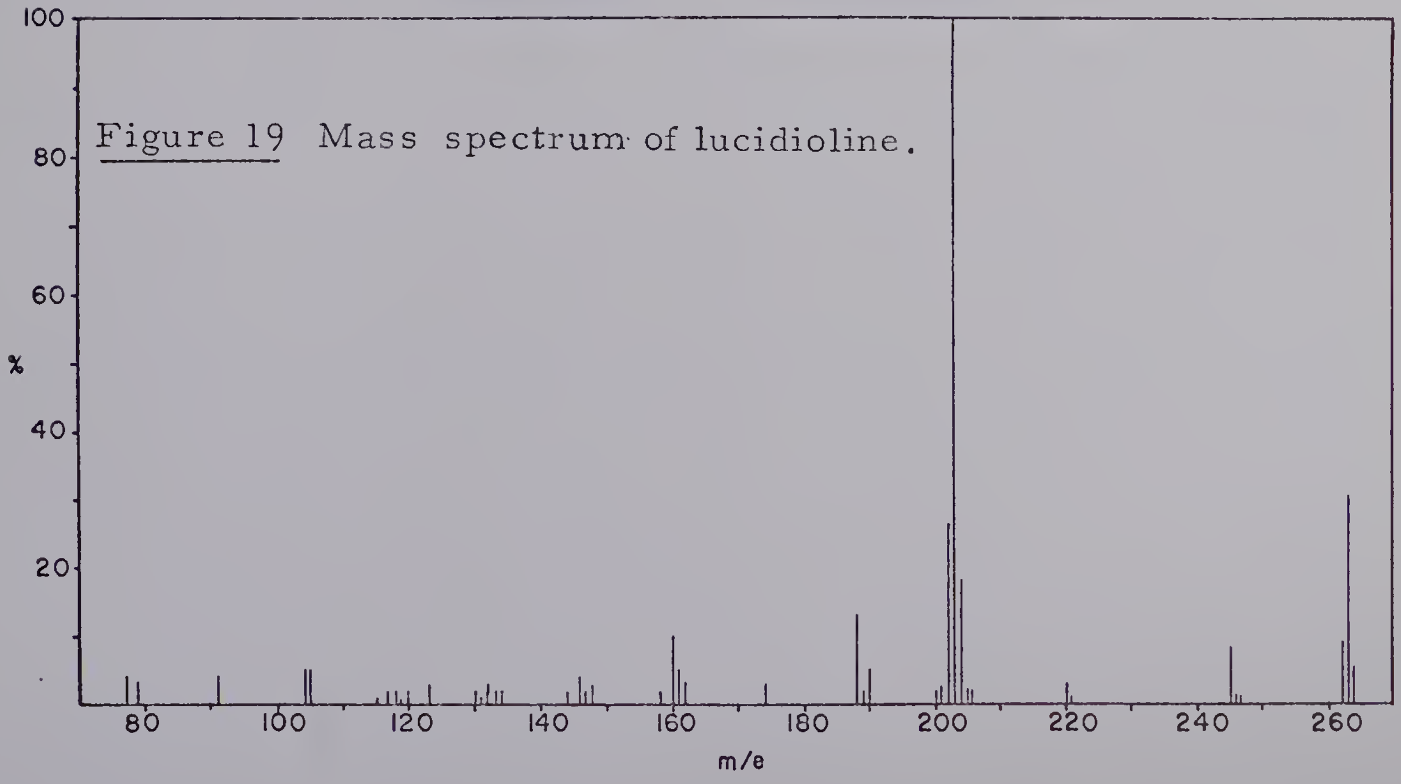
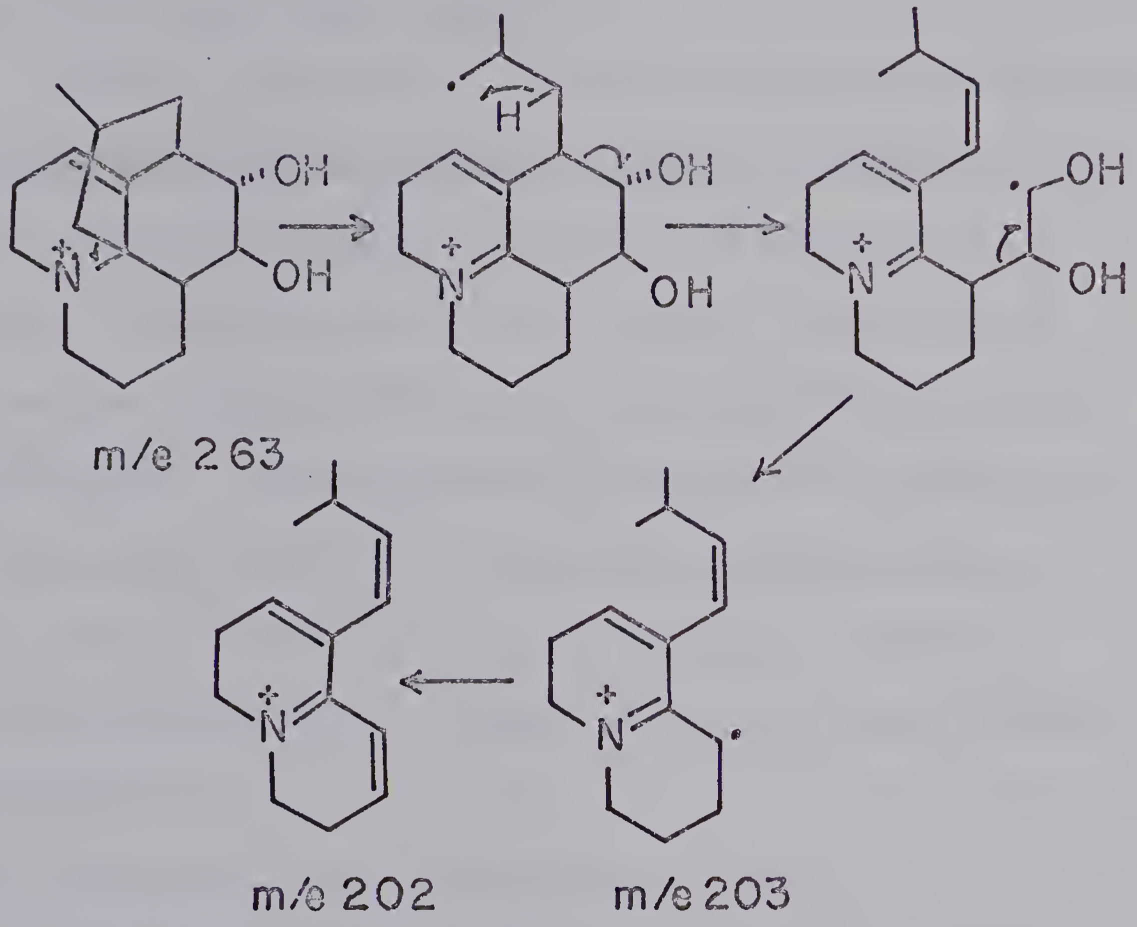


Figure 19 Mass spectrum of lucidioline.

by the base peak (m/e 203). A rationalization of the fragmentation of lucidioline upon electron impact is shown below:


OH
OH
N
H
m/e 263
m/e 202
m/e 203

EXPERIMENTAL

PART II

Isolation of the Total Crude Alkaloids*

Finely ground plant (5.1 kg) was extracted for three days with methanol in a Soxhlet extractor. Most of the methanol was removed by evaporation and the residue digested with aqueous hydrochloric acid (6% by volume) and filtered. The insoluble portion was then digested with hydrochloric acid (10% by volume) and again filtered. The filtrates were combined and washed with ether, then basified to pH 10-11 with ammonium hydroxide and extracted with chloroform. The dried (Na_2CO_3) chloroform extract was evaporated under reduced pressure and gave the total crude alkaloids (55.6 g).

Separation of Alkaloids into Weak and Strong Bases

The total crude alkaloids (55.6 g) were dissolved in aqueous hydrochloric acid (10%) and a saturated solution of disodium hydrogen phosphate was added until the solution had pH 6.5. This solution was extracted several times with methylene chloride. Evaporation of the dried (Na_2CO_3) extract gave the "weak bases" (44 g). The pH of the aqueous solution was then raised to about 10

---

* The isolation was carried out by Mr. J. McCutcheon using L. lucidulum Michx. collected near Fredericton, New Brunswick, by Mr. M. A. Stillwell.

by the addition of ammonium hydroxide. Extraction with chloroform gave the "strong bases" (11.0 g).

Isolation of the Alkaloids from the "Strong Bases"

The "strong bases" (35 g) of L. lucidulum were subjected to a seven funnel countercurrent distribution between chloroform (400 ml fractions, moving phase) and potassium hydrogen phthalate buffer (pH 5) (400 ml). After completion of the distribution, each fraction was made strongly basic (pH > 11) with ammonium hydroxide and extracted into chloroform: fraction 1, 4.3 g; fraction 2, 4.1 g; fraction 3, 5.6 g; fraction 4, 2.6 g; fraction 5, 1.6 g; fraction 6, 3.0 g; fraction 7, 13 g. Total of 34.2 g recovered.

Fraction 3 was subjected to chromatography over alumina (activity II-III, 170 g). Elution with benzene (200 ml) and benzene-ether (1:1, 500 ml) gave after evaporation of the solvents lycopodine (2) (0.4 g) identical with an authentic sample. Elution with ether (500 ml) gave a mixture (0.23 g) of two components which after recrystallization from acetone melted at 183-184°. The infrared spectrum of this mixture was the same as that of the 1:1 molecular complex of lycopodine and flabelliformine reported by Berezowsky[76]. The components were separated by preparative tlc and found to be identical with authentic samples of lycopodine and flabelliformine (91). Elution with ether-chloroform (500 ml) and chloroform (500 ml) gave crystals after the solvents had been removed by evaporation. These crystals contained two components which were separated by

fractional crystallization. The first compound to crystallize out of the hot acetone solution was shown to be identical with lycodoline (14), mp 180-181°. The other component was obtained from the mother liquor and recrystallized from a small volume of acetone, mp 163-164°, both alone and when admixed with an authentic sample of alkaloid L.23 (98a) provided by L. Marion. The infrared spectra of the two were superimposable. An analytical sample of alkaloid L.23 was prepared by recrystallization from acetone, mp 163.5-164°. Calcd for $C_{16}H_{25}NO_2$: molecular weight 263.1885. Found: 263.1888 (mass spectrometry); $[\alpha]_D^{26}$ -43° (c, 0.13, EtOH); ir (Nujol) 3530, 1682, and 1405 cm^{-1}; ir ($CHCl_3$) 3510, 2870, 2820, 2760, 2680 ("Bohlmann bands"), 1690, and 1410 cm^{-1}; nmr ($CDCl_3$) τ 6.41 (1H, s, absent in presence of D_2O) and 9.03 (3H, poorly resolved doublet); mass spectrum m/e 152 (67); 190 (100); 192 (45); 206 (33); 218 (25); 220 (36); 235 (21, $C_{15}H_{25}NO_2$), 246 (76); 263 (100, $C_{16}H_{25}NO_2$), prominent metastable peaks at m/e 230, 210 and 184.

Alkaloid L.23 hydroperchlorate was prepared in the usual way and recrystallized from acetone-methanol, mp 287-290° (dec, literature value 300°)[70]; ir (Nujol) 3450, 3100, 1700, and 1405 cm^{-1}.

Elution with chloroform-methanol (4:1) gave a fraction which when evaporated gave crystals that were not further purified; ir (Nujol) 3200, 1665, and 1630 cm^{-1}; uv (MeOH) 280 mμ (ε 5,000); uv (MeOH-NaOH) 335 mμ (ε 3,000). The infrared and ultraviolet

spectra of this very polar compound were identical with the corresponding spectra of the diosphenol (92) from alkaloid L.20 prepared by Law[77].

Chromatography of countercurrent distribution fraction 6 (3.0 g) over alumina (100 g) and elution with benzene-ether (250 ml) gave on evaporation of the solvent a mixture of two alkaloids (0.38 g). This mixture was dissolved in methanol (5 ml), sodium borohydride (0.2 g) added and the solution allowed to stand overnight. The methanol was evaporated and the residue dissolved in water and extracted with chloroform. The dried ($MgSO_4$) chloroform solution was evaporated and the residual oil (0.35 g) adsorbed on basic alumina (10 g). Elution with benzene (50 ml) gave, on evaporation of the solvent, crystals, mp 117-118° both alone and when mixed with an authentic sample of lycodine (5). Comparison of infrared spectra confirmed the identity. Elution with ether gave on evaporation of the solvent a solid which was recrystallized from ether, mp 167-168°. This compound was shown by mixture melting point and comparison of infrared spectra to be identical with dihydrolycopodine (17) prepared by Law[77].

Further elution of fraction 6 with chloroform-methanol gave on evaporation of the solvent a compound (32 mg), called lucidioline (119a), mp 230-233°. Lucidioline was found by mixture melting point and comparison of infrared spectra to be identical with a compound previously obtained by Law[78] from L. clavatum var. megastachyon. An analytical sample of lucidioline was

prepared by recrystallization from methanol. Calcd for $C_{16}H_{25}NO_2$: molecular weight 263.1885. Found: 263.1885 (mass spectrometry); ir (Nujol) 3400, 3100, and 1678 cm^{-1}, no carbonyl; nmr (CD_3COOD) τ 4.37 (1H, d, J = 5 cps, CH=C) and 9.05 (3H, d, J = 6 cps, $C\underline{H}_3\text{-}\overset{|}{C}H$); mass spectrum *m/e* 160 (10), 188 (13), 202 (26, $C_{14}H_{20}N$), 203 (100, $C_{14}H_{21}N$), 245 (8), 263 (30, $C_{16}H_{25}NO_2$), metastable peaks at *m/e* 156.8 and 174.5.

Lucidioline hydroperchlorate was prepared in the usual way and recrystallized from methanol, mp 238-240° (dec).

Attempted Acetylation of Alkaloid L.23 (98a)

Alkaloid L.23 (30 mg) was dissolved in pyridine (1 ml). Acetic anhydride (1 ml) was added and the temperature of the solution was kept at 98° for 17 hours. The solvent was removed under reduced pressure and the residue dissolved in chloroform. The chloroform solution was washed with aqueous sodium hydroxide (5%), dried ($MgSO_4$) and evaporated. The crystalline residue was recrystallized from acetone (21 mg), mp 162-164° unchanged on admixture with alkaloid L.23. The R_f value and the infrared spectrum confirmed the identity of this compound.

Dehydration of Alkaloid L.23 (98a)

Alkaloid L.23 (60 mg) was dissolved in pyridine (4 ml) containing phenylphosphonic dichloride (4 ml) and the solution was heated to 60° for 17 hours. The reaction mixture was cooled and poured onto ice. The resulting solution was made basic by the addition of sodium hydroxide pellets and then extracted several

times with chloroform. Evaporation of the dried ($MgSO_4$) chloroform left an oil (40 mg) which showed two spots on tlc, one corresponding to starting material and the other to anhydrolycodoline (25). Chromatography over alumina and elution with benzene gave an oil (ca. 2 mg) which with hydrogen chloride in methanol gave crystals of anhydrolycodoline hydrochloride, mp 273-275° (dec). The infrared spectrum was identical with that of an authentic sample. The ORD spectrum of the free base was virtually identical with that of anhydrolycodoline prepared by dehydration of lycodoline[25].

Further elution of the chromatogram with benzene-ether and ether gave first a mixture of anhydrolycodoline and alkaloid L.23 (ca. 10 mg) and then alkaloid L.23 (20 mg).

The dehydration was also accomplished by heating alkaloid L.23 in toluene in the presence of phosphorus pentoxide. In this case, the yield of anhydrolycodoline was again poor and very little starting material was recovered.

Hydrogenation of Lucidioline (119a)

Lucidioline (50 mg) in methanol (50 ml) was hydrogenated over Adams' catalyst (25 mg) at 50 psi for 3 hours. After the catalyst and solvent were removed the product (50 mg) solidified. Thin-layer chromatography indicated the presence of a major and a very minor component. The major component (40 mg) was isolated by crystallization from acetone-methanol and after sublimation (140°, 0.5 mm Hg) melted at 247-251°; ir (Nujol) 3420 cm^{-1}, no carbonyl, no double bond; mass spectrum m/e 205 (21), 208 (100), 222 (8),

248 (4), and 265 (15, parent peak). Calcd for $C_{16}H_{27}NO_2$: molecular weight 265. This compound (108a) was further characterized as its diacetyl derivative (below).

The mother liquors from the crystallization were separated by preparative tlc. The minor component could not be obtained in crystalline form. Its tlc behaviour was identical with that of desacetyllycoclavine (107a).

Hydrogenation of Lucidioline Hydroperchlorate

Lucidioline (119a) (47 mg) in methanol (50 ml) containing 70% perchloric acid (2 drops) was hydrogenated over Adams' catalyst (25 mg) at 50 psi for 3 hours. After the catalyst was removed and the solution concentrated, aqueous ammonia was added and the solution extracted with chloroform. Evaporation of the chloroform gave a semicrystalline solid (45 mg) comprising two components in approximate equal amounts (determined by tlc). Separation was achieved by chromatography over basic alumina (2 g) using ethyl acetate (20 ml fractions) as the eluting solvent. Fractions 3 to 5 were homogeneous on tlc and crystallized to give desacetyllycoclavine (107a) (8 mg) identical (mp, mmp, infrared spectrum, mass spectrum, tlc behavior) with an authentic sample[78]. Fractions 6 and 7 (15 mg) contained both components. Fractions 8 to 20 yielded 19 mg of the 12-epi-desacetyllycoclavine (108a) previously obtained by hydrogenation of lucidioline.

Attempted Acetylation of Lucidioline (119a)

Lucidioline (18 mg) was dissolved in pyridine (2 ml).

Acetic anhydride (2 ml) was added and the solution kept at room temperature for 23 hours. The solvents were removed by evaporation and the residue dissolved in chloroform. The chloroform solution was washed with aqueous sodium hydroxide (5%), dried ($MgSO_4$), and evaporated. The residual oil (20 mg) showed two spots on tlc of equal intensity and similar R_f value. Chromatography of this mixture over alumina (1 g) (elution with benzene) was unsuccessful in separating the components. The ir spectrum ($CHCl_3$) of the mixture showed weak hydroxyl absorption, a single carbonyl absorption at 1735 cm^{-1} and a strong "acetate band" at 1230 cm^{-1}. The nmr spectrum ($CDCl_3$) of the mixture showed four C-methyl peaks (τ 9.06-9.14) and three acetate-methyl peaks (τ 7.92-7.98). Signals at τ 4.6, 5.0, 5.25, and 6.2 were also present in the nmr spectrum. These results indicated that the mixture was probably composed of the mono- and di-acetyllucidioline. The mixture was reacetylated under the same conditions for 2 days but the composition remained unchanged.

Acetylation of Dihydrolucidioline (108a)

Dihydrolucidioline (36 mg) was kept in a mixture of pyridine (2 ml) and acetic anhydride (2 ml) at room temperature for 3 days. After workup an oil (44 mg) was obtained. This was purified by chromatography over alumina (1.5 g). Elution with ether gave a tlc homogeneous oil (38 mg) that could not be induced to crystallize. The analytical sample was purified by evaporative distillation. Calcd for $C_{20}H_{31}NO_4$: molecular weight 349.2253.

Found: 349.2246 (mass spectrometry); mass spectrum m/e 190 (44), 230 (100), 290 (53), 292 (56), 306 (8), 349 (13); ir (CCl_4) no OH or NH absorption, 2750 and 2800 ("Bohlmann bands"), and 1735 cm^{-1}; nmr (CCl_4) τ 5.06 (1 H, d, J = 7 cps, $-\overset{|}{C}\underline{H}OAc$), 5.45 (1H, s, $W_{1/2}$ = 3 cps, $-\overset{|}{C}\underline{H}OAc$), 7.98 and 8.02 (two 3-proton singlets, $-OCOC\underline{H}_3$), and 9.08 (3H, d, J = 6 cps, $-\overset{|}{C}HC\underline{H}_3$).

Hydrogenation of Acrifoline (36)

Acrifoline (527 mg) in methanol (50 ml) was shaken over Adams' catalyst (150 mg) at a hydrogen pressure of 50 psi for 3 hours. Filtration of the catalyst and evaporation of the methanol gave a solid which after recrystallization from ether melted at 167-171° (502 mg). Dihydroacrifoline (109a) has previously been reported by Iverach[95] (mp 168-173°). Comparison of the infrared spectra showed them to be identical.

Reduction of Dihydroacrifoline (109a)

Hydrazine which had been refluxed over sodium hydroxide pellets for 3 hours was distilled into a solution of diethylene glycol (25 ml) and sodium (0.5 g). The resulting solution was distilled until the solution temperature reached 190°. The solution was allowed to cool to 50° and dihydroacrifoline(300 mg) was added. The solution was refluxed at a solution temperature of 190-195° for 17 hours. The cooled reaction mixture was diluted with water, acidified with 6N hydrochloric acid, and continuously extracted with ether for 24 hours. The aqueous layer was separated, made basic with concd ammonium hydroxide and extracted with chloroform. The dried

($MgSO_4$) chloroform extract was evaporated to a solid residue (253 mg). Recrystallization from acetone gave crystals, mp 99-100°. Calcd for $C_{16}H_{27}NO$: molecular weight 249.2093. Found: 249.2090 (mass spectrometry); ir (Nujol) 3400 cm^{-1}, no carbonyl; ir ($CHCl_3$) 3620, 2820, 2760 ("Bohlmann bands"), and no carbonyl; nmr ($CDCl_3$) τ 6.1 (1H, m, $-\overset{|}{\mathrm{C}}\underline{\mathrm{H}}\mathrm{OH}$), 6.65 (1H, s, $-\overset{|}{\mathrm{C}}\mathrm{HO}\underline{\mathrm{H}}$), and 9.10 (3H, d, J = 5 cps); mass spectrum *m/e* 137 (45), 190 (13), 192 (100), 193 (58), 206 (35), 232 (8), 249 (44, parent peak).

Oxidation of Dihydroepiallolycopodine (110a)

Dihydroepiallolycopodine (100 mg) was dissolved in acetone (15 ml) and Jones' reagent (1.0 ml) added dropwise. After a few minutes methanol (1 ml) was added and then most of the solvent was removed under reduced pressure. The residue was dissolved in water, concd ammonium hydroxide added and the basic solution (pH > 11) extracted with chloroform. Evaporation of the chloroform left an oil (81 mg) which was dissolved in ether and filtered through a pad of alumina. The resulting tlc homogeneous oil could not be induced to crystallize. Calcd for $C_{16}H_{25}NO$: molecular weight 247.1936. Found: 247.1936 (mass spectrometry); ir ($CHCl_3$) 2815, 2750 ("Bohlmann bands"), and 1700 cm^{-1}, no OH; nmr ($CDCl_3$) τ 9.20 (d, J = 5.5 cps, $-\overset{|}{\mathrm{C}}\mathrm{HC}\underline{\mathrm{H}}_3$), no signals below τ 7.5; mass spectrum *m/e* 137 (28), 162 (21), 176 (9), 190 (100), 191 (20), 204 (15), 232 (3), and 247 (11).

Attempts to prepare a crystalline hydroperchlorate salt of epiallolycopodine (111a) resulted in an oil which could not be

induced to crystallize.

Epiallolycopodine methiodide was prepared from methyl iodide in methanol solution in the usual way, mp 283-284°.

Oxidation of Dihydroacrifoline (109a)

A solution of dihydroacrifoline (530 mg), cyclohexanone (4 ml), aluminum isopropoxide (500 mg), and toluene (30 ml) was refluxed for 2 hours. The reaction solution was diluted with benzene and extracted with 1N hydrochloric acid. The acidic extract was washed with ether, basified with concd ammonium hydroxide, and extracted with chloroform. The dried ($MgSO_4$) chloroform extract was evaporated to yield an oily residue (500 mg) which was chromatographed over basic alumina (15 g). Elution with ether gave a crystalline component (220 mg), mp 135-138°, which had the same infrared spectrum as the diketone (112a) prepared by Iverach[95]; ir ($CHCl_3$) 2760 and 2820 ("Bohlmann bands"), and 1720 cm^{-1}, no OH.

Selective Reduction of Diketone (112a)

The diketone (200 mg) prepared from dihydroacrifoline was dissolved in glacial acetic acid (3 ml). Ethanedithiol (1 ml) and boron trifluoride etherate (1 ml) were added and the solution allowed to stand at room temperature for 36 hours[96]. The liquid was removed by evaporation under reduced pressure and the residue dissolved in methanol. Raney nickel (2 g, W-2) was added and the solution refluxed for 12 hours. The catalyst was removed by filtration and the solvent by evaporation. The residue (186 mg) was dissolved in acetone. Jones' reagent (1 ml) was added

dropwise. After a few minutes methanol (1 ml) was added and the solvent removed by evaporation. The residue was dissolved in water. The aqueous solution was made basic with concd ammonium hydroxide and extracted with chloroform. The dried ($MgSO_4$) chloroform extract was evaporated to give an oil (134 mg). This oil was chromatographed over basic alumina (12 g). Elution with benzene first gave an oil (23 mg), devoid of carbonyl absorption in the infrared. This was followed by epilycopodine (15) (25 mg) identified by comparison with an authentic sample[25]. Elution with ether-benzene gave fractions that contained two components (10 mg total) with R_f values corresponding to epilycopodine and starting diketone. Elution with ether gave the starting diketone (30 mg).

Bromination and Hydrolysis of Alkaloid L.23 (98a)

Alkaloid L.23 (100 mg) was dissolved in methanol. Hydrobromic acid (45%) was added until the solution showed red on litmus paper. The solvent was removed and the salt recrystallized from methanol, mp 295-299° (dec); ir (Nujol) 3320, 1695, and 1410 cm^{-1}. Alkaloid L.23 hydrobromide (105 mg) was dissolved in glacial acetic acid (2 ml), phenyltrimethylammonium perbromide (150 mg) added and the solution stirred at room temperature for 12 hours. The solvent was removed by evaporation under vacuum. The residual semisolid was dissolved in aqueous sodium bicarbonate (5%) (50 ml) and the solution stirred for 20 hours. The aqueous solution was extracted with chloroform. The chloroform extract was dried ($MgSO_4$) and evaporated to give a solid (78 mg). Recrystal-

lization from acetone gave crystals, mp 154-156°; ir (CCl_4) 2810, 2740, and 1780 cm^{-1}; nmr (CCl_4) τ 9.03 (d, J = 5.5 cps), no signals below τ 7.4; mass spectrum m/e 160 (33), 161 (60), 162 (57), 174 (100), 216 (20), 217 (16), 260 (33), 261 (48, parent peak). Exact masses were measured of peaks m/e 217.1832 (calcd for $C_{15}H_{23}N$: 217.1831) and 261.1730 (calcd for $C_{16}H_{23}NO_2$: 261.1729).

BIBLIOGRAPHY

1. Bodeker, K., Ann., 208, 363 (1881).
2. Manske, R. H. F., Alkaloids 5, 295 (1955).
3. Manske, R. H. F., Alkaloids 10, 305 (1968).
4. Wiesner, K., Ayer, W. A., Fowler, L. R., and Valenta, Z., Chem. and Ind. (London), 564 (1957).
5. Przybylska, M. and Marion, L., Can. J. Chem., 35, 1075 (1957).
6. MacLean, D. B. and Harrison, W. A., Chem. and Ind. (London), 261 (1960).
7. Inubushi, Y., Ishii, H., Yasui, B., Hashimoto, M., and Harayama, T., Chem. and Pharm. Bull. (Japan), 16, 92 (1968).
8. Yasui, B., Ishii, H., Harayama, T., Nishino, R., and Inubushi, Y., Tetrahedron Letters, 3967 (1966).
9. Ayer, W. A., Jenkins, J. K., Piers, K., Valverde-Lopez, S., Can. J. Chem., 45, 445 (1967).
10. Nkunika, D. S., Ph.D. Thesis, University of Alberta, 1967.
11. Ayer, W. A., Iverach, G. G., Jenkins, J. K., and Masaki, N., Tetrahedron Letters, in press.
12. Ayer, W. A. and Iverach, G. G., Can. J. Chem., 38, 1823 (1960).
13. MacLean, D. B., Can. J. Chem., 41, 2654 (1963).
14. Djerassi, C., J. Am. Chem. Soc., 85, 2470 (1963).
15. Hogg, A. M., Ph.D. Thesis, University of Alberta, 224 (1964).

16. Ayer, W. A., Jenkins, J. K., and Valverde-Lopez, S., Tetrahedron Letters, 2201 (1964).

17. Szarek, W. A., Adams, K. A. H., Curcumelli-Rodostamo, A., and MacLean, D. B., Can. J. Chem., 42, 2584 (1964).

18. Ishii, H., Yasui, B., Harayama, T., and Inubushi, Y., Tetrahedron Letters, 6215 (1966).

19. Inubushi, Y., Ishii, H., Harayama, T., Burnell, R. H., Ayer, W. A., and Altenkirk, B., Tetrahedron Letters, 1069 (1967).

20. Conroy, H., Tetrahedron Letters, No. 10, 34 (1960).

21. Gupta, R. N., Castillo, M., MacLean, D. B., Spenser, I. D., and Wrobel, J. T., J. Am. Chem. Soc., 90, 1360 (1968).

22. Manske, R. H. F., Alkaloids 11, 459 (1968).

23. Gupta, R. N. and Spenser, I. D., Chem. Commun., in press.

24. Wiesner, K., Fortsch. Chem. Org. Naturstoffe, 20, 271 (1962).

25. Ayer, W. A. and Iverach, G. G., Can. J. Chem., 42, 2514 (1964).

26. Anet, F. A. L. and Rao, M. V., Tetrahedron Letters, No. 20, 9 (1960).

27. Ayer, W. A., Berezowsky, J. A., and Iverach, G. G., Tetrahedron, 18, 567 (1962).

28. Ayer, W. A., Law, D. A., and Piers, K., Tetrahedron Letters, 2959 (1964).

29. Ayer, W. A., Berezowsky, J. A., and Law, D. A., Can. J. Chem., 41, 649 (1963).

30. Young, J. C. F. and MacLean, D. B., Can. J. Chem., 41, 2731 (1963).

31. Valenta, Z., Deslongchamps, P., Ellison, R. N., and Wiesner, K., J. Am. Chem. Soc., 86, 2533 (1964).

32. Ayer, W. A. and Piers, K., Can. J. Chem., 45, 451 (1967).

33. Ayer, W. A., Jenkins, J. K., Valverde-Lopez, S., and Burnell, R. H., Can J. Chem., 45, 433 (1967).

34. Bohlmann, F. and Schmidt, O., Chem. Ber., 97, 1354 (1964).

35. Colvin, E., Martin, J., Parker, W., and Raphael, R. A., Chem. Commun., 596 (1966).

36. Stevens, R. V., Ph.D. Thesis, Indiana University (1966).

37. Dugas, H., Ellison, R. A., Valenta, Z., Wiesner, K., and Wong, C. M., Tetrahedron Letters, 1279 (1965).

38. Stork, G., Knetchmer, R. A., and Schlessinger, R. H., J. Am. Chem. Soc., 90, 1647 (1968).

39. Ayer, W. A., Bowman, W. R., Joseph, T. C., and Smith, P., J. Am. Chem. Soc., 90, 1648 (1968).

40. Wiesner, K. and Poon, L., Tetrahedron Letters, 4937 (1967).

41. Wall, M. E., Fenske, C. E., Garvin, J. W., Willamen, J.J., Jones, Q., Schubert, B. G., and Gentry, H. S., J. Amer. Pharm. Assoc., 48, 695 (1959).

42. Valverde-Lopez, S., Ph.D. Thesis, University of Alberta, 140 (1963).

43. Loev, B. and Snader, K. M., Chem. and Ind. (London), 15 (1965).

44. Ayer, W. A., Altenkirk, B., Valverde-Lopez, S., Douglas, B., Raffauf, R. F., and Weisbach, J. A., Can. J. Chem. 46, 15 (1968).

45. Boit, H. G., Ergebnisse der Alkaloid Chemie bis 1960, Akademie-Verlag, Berlin, 1961.

46. Burnell, R. H. and Taylor, D. R., Tetrahedron 15, 173 (1961).

47. Burnell, R. H., J. Chem. Soc., 3091 (1959).

48. Leonard, J. and Gash, V. W., J. Am. Chem. Soc., 76, 2588 (1942).

49. Stork, G., Brizzolara, A., Landesman, H., Szmuszkovicz, J., and Terrell, R., J. Am. Chem. Soc., 85, 207 (1963).

50. Inubushi, Y., Ishii, H., Yasui, B., and Harayama, T., Chem. and Pharm. Bull. (Japan), 16, 101 (1968).

51. Ayer, W. A., Bowman, W. R., Kebarle, P., and Burnell, R. H., Can. J. Chem., 43, 328 (1965).

52. Burnell, R. H. and Mootoo, B. S., Can. J. Chem. 39, 1090 (1961).

53. Ayer, W. A. and Piers, K., Chem. Commun., 1, 541 (1965).

54. Burnell, R. H., Chin, C. G., Mootoo, B. S., and Taylor, D. R., Can. J. Chem., 41, 3091 (1963).

55. Leonard, N. J., Record of Chemical Progress, 17, 243 (1956).

56. Fodor, G. and Nador, K., J. Chem. Soc., 721 (1953).

57. House, H. O., "Modern Synthetic Reactions", W. A. Benjamin, Inc., N. Y., 1965, p. 57.

58. Chapman, J. H., Elks, J., Phillipps, G. H., and Wyman, L. J., J. Chem. Soc., 4344 (1956).

59. Chapman, O. L. and King, R. W., J. Am. Chem. Soc., 86, 1256 (1964).

60. Traynham, J. G. and Knesel, G. A., J. Am. Chem. Soc., 87, 4220 (1965).

61. Scott, A. I., "Interpretation of the Ultraviolet Spectra of Natural Products", Pergamon Press, N. Y., 1964, p. 115.

62. Bellamy, L. J., "The Infrared Spectra of Complex Molecules", John Wiley and Sons, N.Y., 1958, pp. 71 and 179.

63. Budzikiewicz, H., Djerassi, D., and Williams, D. H., "Interpretation of Mass Spectra of Organic Compounds", Holden-Day, Inc., San Francisco, 1964, p. 194.

64. Huitric, A. C., Carr, J. B., Trager, W. F., and Nist, B. J., Tetrahedron, 19, 2145 (1963).

65. Dyer, J. R., "Aplications of Absorption Spectroscopy of Organic Compounds", Prentice-Hall, Inc., Englewood Cliffs, N.J., p. 87.

66. Belleau, B. and McLean, S., in "Elucidation of Structures by Physical and Chemical Methods", Bentley, K. W. ed., Interscience Publishers, 1963, p. 922.

67. Cookson, R. C., Henstock, J., and Hudec, J., J. Am. Chem. Soc., 88, 1060 (1966).

68. Bobbitt, J. M., "Thin-Layer Chromatography", Reinhold Pub. Co., N.Y., 1963, p. 93.

69. Praill, P. F. G. and Whitear, A. L., Proc. of Chem. Soc., 112 (1961).

70. Manske, R. H. F. and Marion, L., Can. J. Res. B. 24, 57 (1946).

71. Ayer, W. A., Altenkirk, B., Burnell, R. H., and Moinas, M., Can. J. Chem., in press.

72. Ayer, W. A., Masaki, N., and Nkunika, D. S., Can. J. Chem., in press.

73. Schrager, I. G. and Rosentsveig, P. E., Farmatseut Zh. (Kiev), 19, 49 (1964); Chem. Abs., 61, 5450 (1964).

74. Achmatowicz, O. and Rodewald, W. J., Roczniki Chem., 30, 233 (1956).

75. Rodewald, W. J. and Grynkiewicz, G., Ann. Soc. Chim. Polonorum, 42, 465 (1968).

76. Berezowsky, J. A., M.Sc. Thesis, University of Alberta, 1962.

77. Law, D. A., Ph.D. Thesis, University of Alberta, 1963.

78. Ayer, W. A. and Law, D. A., Can. J. Chem., 40, 2088 (1962).

79. Bohlmann, F., Chem. Ber., 91, 2157 (1958).

80. Bohlmann, F., Angew. Chem., 69, 641 (1957).

81. Hamlow, H. P. and Okuda, S., Tetrahedron Letters, 2553 (1964).

82. Bohlmann, F., Schumann, D. and Schulz, H., Tetrahedron Letters, 173 (1965).

83. Jackman, L. M., "Application of NMR in Organic Chemistry", Pergamon Press, London, 1959, p. 116.

84. Hogg, A. M., Ph.D. Thesis, University of Alberta, 1964.

85. Ayer, W. A., Habgood, T. E., Deulofeu, V. and Juliani, H. R., Tetrahedron, 21, 2169 (1965).

86. Wiesner, K., Francis, J. E., Findlay, J. A., and Valenta, Z., Tetrahedron Letters, No. 5, 187 (1961).

87. Moffitt, W., Woodward, R. B., Moscowitz, A., Klyne, W., and Djerassi, C., J. Am. Chem. Soc., 83, 4013 (1961).

88. Hohn, E. G. and Wiegang, O. E. Jr., J. Chem. Phys. 48, 1127 (1968).

89. Horeau, A. and Kagan, H. B., Tetrahedron, 20, 2431 (1964).

90. Valverde-Lopez, S., Ph.D. Thesis, University of Alberta, 1966, p. 131.

91. Curcumelli-Rodostamo, M. and MacLean, D. B., Can. J. Chem. 40, 1068 (1962).

92. Anet, F. A. L., Tetrahedron Letters, No. 20, 13 (1960).

93. Barton, D. H. R., Ives, D. A. J., and Thomas, B. R., J. Chem. Soc., 2056 (1955).

94. Djerassi, C., "Organic Reactions", Vol. 6, John Wiley and Sons, N. Y., 1951, p. 207.

95. Iverach, G. G., Ph.D. Thesis, University of Alberta, 1963, p. 68.

96. Fieser, L. F., J. Am. Chem. Soc., 76, 1945 (1954).

APPENDIX 1

Correspondence with Prof. Y. Inubushi

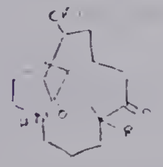
March 28, 1966.

Professor Y. Inubushi,
Faculty of Pharmaceutical Sciences,
Osaka University,
Tayonaka,
Osaka-Fu,
Japan.

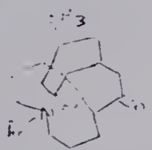
Dear Professor Inubushi:

Thank you very much for your recent letter and for the preprints of your papers on serratinine. I would like to compliment you on the work on serratinine, it is a very good piece of work.

I think it is possible that a compound which we have isolated, alopecuridine (see accompanying preprint) may represent a biogenetic link between lycodoline and serratinine. It is possible that it has structure I (R=OH) and that fawcettimine (Burnell, Can. J. Chem. 41, 3091 (1963)) has structure I (R=H).



I



II

Perhaps it would be possible to correlate fawcettimine and serratinine. Compound II is readily available from fawcettimine, and we shall attempt to transform it into your O,O,N-triacetylchanodihydroserratinine. In connection with this I would very much like to run the mass spectrum of your O,O,N-triacetylchanodihydroserratinine if you could spare a milligram or two.

Congratulations again on a very good piece of work !

Yours sincerely,

W. A. Ayer.

FACULTY OF PHARMACEUTICAL SCIENCES
OSAKA UNIVERSITY
TOYONAKA OSAKA FU, JAPAN

November 4, 1966

Professor W. A. Ayer
Department of Chemistry,
University of Alberta,
Edmonton, Alberta
Canada

Dear Professor Ayer;

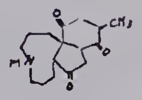
Recently, we have established structures of three alkaloids which had seemed to be closely related to serratinine and I enclose a reprint and two preprints on these works.

In your former letter, you pointed out that it is possible from biogenetic consideration of serratinine type alkaloids we suggested, that fawcettimine has structure (1).

On the basis of your suggestion, we transformed to the compound II, m.p. 205-207°, from serratinine which may correspond to N-acetyldihydrofawcettimine, reported m.p. 205-206°.

I should appreciate if you would mind comparing our sample with N-acetyldihydrofawcettimine from natural alkaloid. If they are identical, I would like to suggest you that I would gladly have a joint paper with you in some journal. I would like to leave your decision on this matter.

I am looking forward to hear from you.

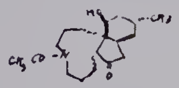


(I)

Sincerely yours,

Yasuo Inubushi
Yasuo Inubushi

compound II.



APPENDIX 2

Alkaloids from L. Phyllanthum

Thin-layer chromatography of the total alkaloids of L. phyllanthum showed that there were about 12 compounds present. A small specimen (90 mg) of the total alkaloids was subjected to elution chromatography on basic alumina (6.0 g) but only impure fractions were obtained. Preparative thin-layer chromatography of these chromatographic fractions did not give pure compounds.

An attempt was made to separate the components of the total alkaloids by glpc*. One alkaloid was separated in a pure form and this was shown to be identical with lycodoline (14) (tlc behaviour and mass spectrum)[25].

A crystalline material (13 mg), mp 250-258°, had been obtained from the total alkaloids during the isolation procedure**. This compound was recrystallized from acetone, mp 258-259°, and shown to be identical with alkaloid L.20 (18) (tlc behaviour, ir and mmp)[29].

---

* This work was done by Mr. D. McMinn.

** Total alkaloids of L. phyllanthum isolated by Smith Kline and French Laboratories, Philadelphia, U. S. A..

APPENDIX 3

Alkaloids from Unnamed Lycopodium species (Peru)

Preparative thin-layer chromatography separated the total alkaloids (13 mg) of this unnamed Lycopodium species from Peru* into two main components. The first component (2.1 mg) was purified by molecular distillation and was shown to be identical with cernuine (22) (tlc behaviour, ir and mass spectrum)[33].

The other component (2.1 mg) had an infrared spectrum very similar to lycocernuine (23). This was confirmed by the preparation of O-acetyllycocernuine with acetic anhydride in pyridine solution. The acetylated material was purified by molecular distillation and gave a mass spectrum identical with the spectrum of O-acetyllycocernuine[33].

---

* Total alkaloids isolated by Smith Kline and French Laboratories, Philadelphia, U. S. A..

The University of Alberta
Printing Department
Edmonton, Alberta

B29914